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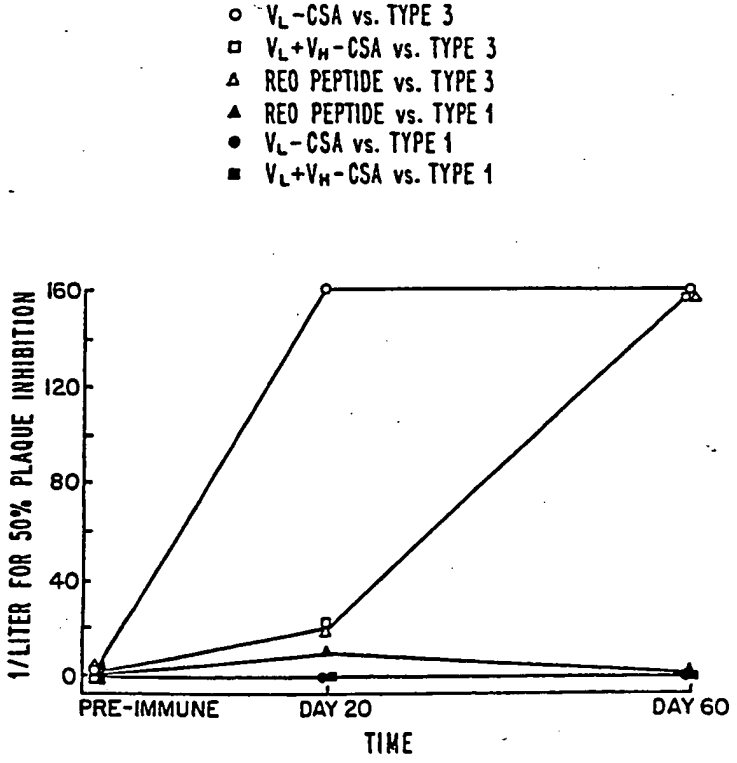
(57) Abstract

A novel method of synthesizing immunogens is disclosed. First a shared sequence between an anti-receptor antibody for an external antigen and antigen itself is determined. The shared sequence is then used to construct peptides which may be used as immunogens. A method of providing agents for binding to cellular receptors is also disclosed. Such receptor binding agents are similarly constructed by determining a shared sequence between anti-receptor antibodies and an external antigen. The resulting products may be used as vaccines or as pharmaceuticals which are either directly active on the target cells, or indirectly active as the result of preventing receptor binding by the subject antigen.



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IMMUNOGENS AND BIOLOGICALLY ACTIVE PEPTIDES DERIVED FROM SHARED SEQUENCES FROM ANTIGENS AND ANTI-IDIOTYPIC ANTIBODIES OR ANTIBODIES SPECIFIC FOR CELLULAR RECEPTORS OF THE ANTIGENS

CROSS-REFERENCE TO RELATED PATENTS

The present application is a continuation-in-part of U.S. patent application 074,264 filed July 16, 1987 in the name of Mark I. Greene. The present application is related to U.S. Application 194,026 filed May 13, 1988 in the names of Mark I. Greene, William V. Williams, David B. Weiner and Jeffrey A. Cohen, assigned to University Patent, Inc. and to U.S. patent 4,490,358, filed in the names of Mark I. Greene and Bernard N. Fields, entitled "Screening Vaccines and Immunization Process", assigned to the President and Fellows of Harvard College, which application and patent are hereby incorporated by reference as if fully set forth herein.

BACKGROUND OF THE INVENTION

The present invention relates to the field of agents which are useful in the immunization of mammals against infectious organisms, such as bacteria, fungi, parasites, and viruses and neoplasms which express specific antigens. Such organisms typically have specific sites which bind to a complementary portion, called a receptor site, of the host cells. This invention also relates to the field of biologically active agents, such as pharmaceuticals, which act on mammalian cells by binding to the r ceptor site(s) of those cells to alter or affect their function or behavior, or to

prevent or alter the effect which another agent, such as an antigen or pathogen, would otherwise have upon those cells.

Several strategies have been employed to develop safe, effective vaccines against viral and bacterial pathogens. At present most vaccines in use consist of live attenuated pathogens, while inactivated pathogens, components of a pathogen, or modified toxins (toxoids). See Institute of Medicine, "Vaccine Supply and Innovation", Washington, D.C.: National Academy Press (1985). While these preparations have been successfully used for many infectious diseases, many pathogens exist where these approaches have not worked or have not been applicable. Certain pathogens are potentially too dangerous to contemplate the use of attenuated or even inactivated preparations. The risk of developing cancer from immunization with certain retroviruses, or of developing acquired immunodeficiency syndrome (AIDS) from immunization with human immunodeficiency virus (HIV) underscores the drawbacks associated with the use of whole virus preparations for vaccination. In addition many pathogens display a marked antigenic heterogeneity that makes effective vaccination difficult. These considerations have led us to seek alternative method for effective immunization.

The idiotype network theory of N.K. Jerne, "Towards a Network Theory of the Immune System", Ann. Immunol. (Paris) 125: 337-389, (1974) implies that an anti-idiotypic antibody raised against a neutralizing antibody specific for a pathogen would mimic that pathogen immunologically. Immunization with the anti-idiotype should result in the development of a significant anti-pathogen response with the elicitation of neutralizing antibodies and cell-mediated immunity. In recent years there have been several examples where this strategy has been effective, including reovirus type 3. See Sharpe, A.H., Gaulton, G.N., McDade, K.K., Fields, B.N., and Greene, M.I., "Syngeneic Monoclonal Antiidiotype Can Induce Cellular Immunity to Reovirus", J. Exp. Med., 160: 195-205 (1984); Kauffman, R.S., Noseworthy, J.H., Nepom, J.T., Finberg, R., Fields, B.N., and Greene, M.I., "Cell Receptors for Mammalian

Reovirus II: Monoclonal Anti-Idiotypic Antibody Blocks Viral Binding to Cells", J. Immunol., 131:2539-2541, (1983); and Gaulton, G.N., Sharpe, A.H., Chand, D.W., Fileds, B.N. and Greene, M.I. (1986). "Syngeneic monoclonal internal image anti-idiotopes as prophylactic vaccines". J. Immunol. 137: 2930-2936. With respect to Sendai virus see Ertl, H.C. and Finberg, R.W., "Sendai Virus Specific T Cell Clones: Induction of Cytolytic T Cells by an Anti-Idiotypic Antibody Directed Against a Helper T Cell Clone", Proc. Natl. Acad. Sci. USA, 81:2850-2854, (1984). For report relating to rabies see Reagen, K.J., Wanner, W.H., Wiktor, T., and Koprowski, H., "Anti-Idiotypic Antibodies Induce Neutralizing Antibodies to Rabies Virus Glycoproteins", J. Virol., 48:660-666, (1983). This approach has been discussed in connection with polio virus in Uydeltaag, F.G.C.M. and Osterhaus, A.D.M.E., "Induction of Neutralizing Antibody in Mice Against Polio Virus Type II with Monoclonal Antiidiotypic Antibody", J. Immunol., 134:1225-1229, (1985). One of the key aspects of this approach is that the anti-idiotype mimics a neutralizing epitope on the pathogen and induces a neutralizing response. Thus a potent anti-idiotype vaccine would seem to be an ideal immunogen in cases where intact pathogen could not be used or where irrelevant non-neutralizing epitopes dominate the immune response. However, the practical application of anti-idiotypes as vaccine has been limited by the difficulties in making human monoclonal antibodies and in the danger of producing serum sickness by using xenogeneic antibodies.

Another method currently under intensive investigation is the use of synthetic peptides corresponding to segments of the proteins from pathogenic microorganisms against which an immune response is directed. This approach has been successful in several instances including feline leukemia virus (Elder, J.H., McGee, J.S., Munson, N.M., Houghten, R.A., Kloetzer, W., Bittle, J.L., and Grant, C.K.," Localization of Neutralizing Regi ns f the Envelope Gene of Feline Leukemia Virus by Using Anti-Synthetic Peptide

Antibodies", J. Virol., 61:8-15, 1987), hepatitis B (Gerin, J.L., Alexander, H., Shih, J.W., Purcell, R.H., Dapolito, G., Engle, R., Green, N., Sutcliffe, J.G., Shinnick, T.M., and Lerner, R.A., "Chemically Synthesized Peptides of Hepatitis B Surface Antigen Duplicate the D/Y Specificities and Induce Subtype-Specific Antibodies in Chimpanzees", Proc. Natl. Acad. Sci. USA. 80:2365-2369, 1983), Plasmodium falciparum (Cheung, A., Leban, J., Shaw, A.R., Merkli, B., Stocker, J., Chizzolini, C., Sander, C., and Perrin, L.H., "Immunization With Synthetic Peptides of a Plasmodium Fulciparum Surface Antigen Induces Antimerozoite Antibodies", Proc. Natl. Acad. Sci. USA, 83: 8328-8332, 1986), cholera toxin (Jacob, C.O., Grossfeld, S., Sela, M., and Arnon, R., "Priming Immune Response to Cholera Toxin Induced by Synthetic Peptides", Eur. J. Immunol., 16:1057-1062, 1986) and others. When these peptides are capable of eliciting a neutralizing immune response they appear to be ideal immunogens. They elicit a specific response and typically do not lead to deleterious effects on the host. However, it can be difficult to predict which peptide fragments will be immunogenic and lead to the development of a neutralizing response. It would be desirable to develop immunogens that elicit a response to specific neutralizing epitopes without causing responses to extraneous epitopes that could "dilute" the specific response or lead to harmful immune complex formation.

SUMMARY OF THE INVENTION

The present invention provides novel methods for determining the desirable amino acid sequences of biologically active peptides to thereby provide immunogens capable of eliciting responses to specific neutralizing epitopes without causing response to extraneous epitopes that could "dilute" the desired response or lead to harmful immune complex formation. The invention is predicated upon the surprising discovery that antibodies specific for a cellular receptor of an antigen exhibit a peptide sequence hom 1 gy to the peptide sequence found in the corresponding region of the antigen itself. Accordingly, instead of using anti-receptor antibody

itself as the vaccine or the like, it is used only to identify the shared peptide sequences between corresponding regions of the antigen and anti-receptor antibody. A peptide comprising that shared sequence is then synthesized for therapeutic use.

The present invention thus provides a novel synthetic biologically active peptide comprising a peptide sequence found in both an antigen and an antibody specific for a cellular receptor for that antigen. In the preferred embodiment, the antigen is a virus such as a reovirus, and the peptide comprises at least sixty, preferably eighty to one hundred percent of the amino acids found in an antigen region and the corresponding region of the anti-receptor antibody. Normally, the corresponding region of the anti-receptor antibody is within the complementary determining region of that antibody. It is preferred to use at least a six amino acid sequence from the complementary determining region of and said anti-idiotypic antibody. The complementary determining region normally located on the light chain of the anti-receptor antibody, the CDR II region being particularly preferred.

The present invention further provides a novel method of immunizing a host mammal against an infectious organism which has a site which binds specifically to a receptor site on a host cell. The method comprises the step of inoculating the mammal with a synthetically biologically active peptide having a peptide sequence corresponding to a peptide sequence found in both said site of said infectious organism and in an antibody specific for a receptor on a host cell, in an amount effective to reduce the likelihood that said host will be susceptible to infection by said organism. This method is particularly suited to immunizing a host mammal against infections caused by a virus, such as a reovirus.

Demonstration of the feasibility and utility of the methods and products of the present invention has been accomplished using reovirus 3, a virus which is known to specifically bind to a structure physically similar to the beta-adrenergic receptor of mammalian cells to alter the

metabolic state of those cells. Accordingly, a method f treating a mammal to alter the metabolic state of that mammal is also provided by the present invention. The method comprises the steps of (a) selecting a known substance having a proteinaceous binding site thereon which binds specifically to a receptor site on host cells of said mammal, said substance being known to affect the metabolic state of said cells when bound thereto; (b) providing an antibody specific for said receptor site on host cells of said mammal; (c) comparing corresponding regions of the proteinaceous binding site of said substance and of the binding site of said anti-receptor antibody to locate portions of said binding sites exhibiting at least a sixty percent correspondence in amino acid sequence to thereby identify a shared sequence region; (d) constructing a synthetic peptide comprising at least a portion of said shared sequence region; and (e) administering said synthetic peptide to said mammal in an amount sufficient to alter the metabolic state of said host cells. This method is particularly useful when the subject known substance is an organism such as a virus. Preferably the corresponding region of the anti-receptor antibody is a complementary determining light chain region, such a the CDR II region, having at least six amino acids in shared sequence.

The present invention further provides novel methods for synthesizing biologically active compounds. These methods comprise the steps of (a) selecting a pathogen gene product known to bind to a cellular receptor of mammalian cells; (b) providing an antibody specific for said cellular receptor for said pathogen gene product; (c) comparing the peptide sequences of said gene product and of said anti-receptor antibody to determine at least shared sequence potions thereof; and, (d) synthesizing a compound comprising a biologically active site corresponding to the tertiary structure of said shared sequence portions. The thus synthesized peptide at least comprises, and preferably consists essentially of, at least six amino acids of the shared sequence. Preferred gene products selected in

accordance with step (a) include reovirus gene products, such as those of reovirus 3; vaccinia gene products; Epstein-Barr virus gene products; and gene products which bind to the mammalian beta-adrenergic receptor family.

Novel methods of altering the growth of mammalian cells are also provided by the present invention. These methods include the step of administering to mammalian cells a peptide comprising a sequence of amino acids which is shared by a pathogen gene product known to bind to the beta-adrenergic receptor of that cell and by an antibody specific for said beta-adrenergic receptor, said sequence being selected and said peptide being administered in an amount effective to alter the growth of said cells. The preferred peptides and pathogen gene products for use in these methods are those as described above.

An alternate method for synthesizing biologically active compounds is disclosed which similarly takes advantage of the surprising finding that amino acid sequences are shared between certain pathogen gene products and antibodies specific for a cellular receptor of the antigen (i.e., and anti-receptor antibody). The method again comprises selecting a gene product, as described above, known to bind to a cellular receptor of mammalian cells, and providing an antibody specific for said cellular receptor. The amino acid sequence of the anti-receptor antibody is then determined in a complementary determining region of said antibody, preferably the light chain CDR II portion of that antibody, and a compound comprising a site corresponding to the tertiary structure of a complementary determining region of said anti-receptor antibody is synthesized. The synthesis step of this method may comprise the synthesis of a peptide comprising the shared amino acid sequence comprising at least six amino acids, or other synthetic techniques may be used to provide a compound have an equivalent tertiary structure. The specific peptides so produced may then be bound to suitable carrier proteins to provide a complex which covalently binds to the target receptor. In the preferred embodiment the method

further comprises the step of determining the relative biologic activity of the resultant compound, by preferably determining the relative affinity of the compound for the receptor. This determination may be accomplished by utilizing the ability of the resultant compound to induce the production of antibodies to the pathogen as an indication of relative affinity. Induced immunity to the pathogen is another suitable method for determining the relative biologic activity of the compound which is the product of this method.

Accordingly, a primary object of the present invention is the provision of novel, biologically active peptides.

Another object of the present invention is the provision of novel methods for producing such peptides and/or for determining the tertiary and/or chemical structures of peptides or equivalent compounds which exhibit biologic activity.

A further object of the present invention is the provision of new methods for immunizing mammals against pathogenic organisms and agents, particularly viruses such as reoviruses.

These and other objects of the present invention will become apparent from the following, more detailed description.

BRIEF SUMMARY OF THE FIGURES

Figure 1 illustrates the specific binding of 9BG5 to peptides, determined by radioimmunoassay as noted in the experimental procedures described hereinafter; CPM of 9BG5 bound to blank wells was subtracted from CPm of 9BG5 bound to peptide coated wells; non-specific binding to peptides was corrected for by subtracting from the value a similar value determined for an isotype-matched control monoclonal antibody UPC10; specific CPM of 9BG5 bound to peptide coated wells is shown using the amount of 9BG5 added to each well in a final volume of 50 ul.; mean ± SD for duplicate wells is shown.

Figures 2 and 3 illustrate the binding of V_L-BSA to type 3 reovirus receptor as determined by its ability to compete for binding with anti-reovirus type 3 receptor antibody 87.92.6.; R1.1 cells (10^7/ml) were incubated in 1% BSA in the presence or absence of 200 ug/ml V_L-BSA or V_H-BSA as indicated for 45 minutes; monoclonal antibodies were added at the concentrations noted for an additional 30 minutes; the cells were washed twice and a 1:200 dilution of FITC-goat anti-mouse Fab was added for 30 minutes; the cells were washed twice and analyzed for fluorescence intensity on a FACS analyzer; percent maximal cell staining was determined as the ratio of the percent of the cells positive on FACS analysis at the antibody concentration noted to the maximal percent of cells judged positive at saturating doses of monoclonal antibody in the absence of competitors ([% positive at concentration divided by maximal % positive] X 100); the maximal percent positive values were as follows: 2 - 15.3%, 3 - 97%, 3 - 24%.

Figures 4-7 illustrate specific binding of immune serum to virus-coated plates, determined by radioimmunoassay as noted in the hereinafter described experimental procedures; CPM of immune serum binding to blank wells was subtracted from CPM binding to virus coated wells; to account for non-specific binding to virus coated wells, a similar value determined for normal mouse serum was subtracted form the value determined for immune serum; specific CPM bound is shown versus the

dilution of mouse serum added in a final volume of 50 ul.; the mean ± SEM of duplicate wells from groups of 3 or 4 mice is shown at each dilution.

Figures 8 and 9 illustrate immune serum assays for viral neutralization as described in the following section; serum was collected prior to immunization with peptides (pre-immune or day 0), on day 20 following the first immunization, and on day 60; the neutralization titer was determined at each time point from groups of 4 mice; the geometric mean divided by SEM of the reciprocal of the neutralization titer is shown at each time point.

Figures 10 and 11 illustrates plaque inhibition, determined as indicated in the following description; plaque numbers were determined for 4 mice in each group and the mean values determined; the highest dilution of serum that produced 50% or greater plaque inhibition was determined and is shown for each time point at which serum was obtained; plaque inhibition of both type 1 and type 3 virus is shown.

Figure 12 illustrates data for mice immunized with the reovirus types noted by injection of 10^7 PFU subcutaneously, or with the peptides noted at a dose of 100 ug split into two injections subcutaneously; one week later, mice were challenged with virus or peptides in the footpads; footpad swelling was determined as indicated in the following description 48 hours after challenge; the mean ± SEM for groups of mice is shown.

Figures 13 and 14 shows the delayed type hypersensitivity (DTH) response of mice to intact reovirus type 3 after immunization with peptides.

Figure 15 shows reovirus type 3 and 87.92.6 antibody inhibition of L cell proliferation.

Figure 16 shows inhibition of L cell proliferation by peptides.

Figures 17-20 show modulation of reovirus type 3 receptor by peptides.

Figure 21 shows modulation of the reovirus type 3 receptor by peptides and antibody.

Figures 22 and 23 show inhibition of lymphocyte proliferation.

Figures 24 and 25 show peptide inhibition of con A induced lymphocyte proliferation.

Figures 26 and 27 show competition of binding of 9BG5 antibody to 87.92.6 antibody coated wells in the presence of peptide inhibitors.

Figure 28 and 29 show V_L and variant peptide inhibition of binding of reovirus type 3 particles to 9BG5.

Figures 30-33 show in (a) and (b) V_L peptide inhibition of binding of reovirus type 3 and variant K to L cells; (32) and (33) show V_L variant peptide inhibition of binding of reovirus type 3 to murine L cells.

Figure 34 shows a representational diagram of two alternate routes for the development of biologically active peptides according to the methods of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The preferred embodiments of the present invention are described in connection with experiments which have been conducted using reovirus types 1 and 3 interactions with cellular receptors using the anti-idiotype approach. These example(s) were performed using the following experimental procedures, unless otherwise noted.

Experimental Procedures

Mice

Adult Balb/c female mice, 6 to 8 weeks to age, were obtained from Jackson Laboratories, Bar Harbor, ME. Pre-immune serum was obtained on all mice used and assayed by neutralization of reovirus infectivity (see below) to ascertain that there had been no prior exposure to reovirus. Mice immunized with peptides were housed in the animal care facility and fed a house diet ad libitum (Purina, St. Louis, MO). Mice immunized with reovirus type 3/Dearing were housed in a separate facility.

Viruses

Reovirus type 1 (Lang), and reovirus type 3 (Dearing) and the reassortants 3.HA-1 and 1.HA-3 have been previously described (Fields, B.N. and Greene, M.I., "Molecular Mechanism of Viral Pathogenesis: Implications for Prevention and Treatment", Nature, 20:19-23, 1982). Clones 1.HA-3 and 3.HA-1 are single segment reassortant clones that segregate the S1 gene, the gene encoding the viral attachment polypeptide (hemagglutinin) sigma 1. For mouse inoculation and virus neutralization, a stock of reovirus that was passed twice in L-cells was purified by substituting ultrasonic disruption (Branson Ultrasonic 200) for cell homogenization in a modification of published techniques (Joklik, W.K., "Studies on the Effect of Chymotrypsin on Reovirions", Virology, 49:700-715, 1972). The number of particles per ml was determined by optical density at 260 nm (Smith, R.E., Zweernik, H.J., and Joklik, W.K., "Polypeptide Components of Virions, top Component and Core of Reovirus Type 3", Virology, 39:791-810, 1969).

Monoclonal Antibodies

Type 3 reovirus neutralizing monoclonal antibody 9BG5 (mouse IgG2aK) (Burstin, S.J., Spriggs, D.R., and Fields, B.N., "Evidence for Functional Domains on the Reovirus Type 3 Hemagglutinin", Virology, 117:146-155, 1982) was purified from hydridoma supernatant with the cells grown in Dulbecco's minimal essential media (DMEM) (MA Bioproducts, Walkersville, MD) with added penicillin/streptomycin solution (The Cell Center, University of Pennsylvania, Philadelphia, PA), and 10% fetal bovine serum (FBS). Culture supernatants were precipitated with 50% $(NH_4)_2SO_4$, solubilized in distilled water and dialyzed against 3 changes of phosphate buffered saline (PBS). Next the antibody was purified on a Sepharose-protein A column and eluted with 0.1 M citric acid pH 4.5. The eluate was collected in 1 M tris buffer, pH 8.5 to neutralize excess acidity and dialyzed against 3 changes of PBS. The dialysate was concentrated on an Amicon protein concentrator with a molecular weight cut-off of 30 kilodaltons

(KD). The purified antibody was more than 95% pure by sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Irrelevant monoclonal antibodies UPC-10 and A11 (both mouse IgG2aK) was similarly purified from clarified ascites (Gibco, Grand Island Biological Co.).

Monoclonal antibodies 87.92.6 (mouse IgM,K) and HO 13.4 (mouse IgM,K,anti-Thy 1.2) and HO 22.1 (mouse IgM, K, anti Thy 1.1) were purified from 50% ammonium sulfate cuts of culture supernatant or from ascites supernatants from ascites generated in hydridoma bearing Balb/c mice. These preparations were dialyzed against 3 changes of PBS and run over a goat anti-mouse IgM Affigel-10 column. Antibodies were eluted with 3.5 M $MgCl_2$, dialyzed against 3 changes of PBS and concentrated as noted above. Purity of all monoclonal antibodies used was greater than 95% by SDS-PAGE.

Cell Lines

Murine L-cells were grown in spinner bottles with Joklik's MEM (MA Bioproducts) with 5% FBS. R1.1 cells (murine thymoma, Thy 1.2+) were grown in suspension in RPMI 1640 (MA Bioproducts, Walkersville, MD) supplemented with L-glutamine, 10 mM HEPES buffer (MA Bioproducts) and penicillin/streptomycin with 10% FBS.

Immunization of Mice

For the study of DTH response, groups of mice were inoculated with either synthetic peptide or live reovirus type 3 subcutaneously (s.c.) in 2 separate sites on the dorsal flanks of the animal (over each hind limb); 50 ug of a synthetic peptide or 10^7 viral particles/0.2 ml were given in separate injections of 0.1 ml vol. Six days later, animals were challenged in the left footpad with $3x10^7$ viral particles suspended in saline containing 2% gelatin (30 ul). Footpad swelling was recorded 24 hr later in a blind fashion (Greene, M.I. and Weiner, H.L., "Delayed Hypersensitivity in Mice Infected with Reovirus, II. Induction of Tolerance and Suppr ssor T Cells to Viral Specific Gene Products", J. Immunol., 125:283-287, 1980). Four animals per group were studied, and the magnitude of the response was determined by

comparing the challenged left fo tpad to the untreated right footpad.

For the study of humoral immune response, mice were inoculated with either synthetic peptide or live reovirus type 3 as above with the following modification. The peptide was conjugated with chicken serum albumin (CSA) as described below and 100 ug of the peptide conjugate was inoculated s.c. in 2 divided doses. For mice immunized with synthetic peptides, the first immunization was with peptide mixed with an equal volume of complete Freund's adjuvant; whereas with subsequent immunization the peptide was suspended in saline containing gelatin. Mice were immunized weekly for five weeks, and serum was obtained prior to the first inoculation, and then at the second and sixth week. For mice immunized with reovirus type 3, 10^7 plaque forming units (PFU) was inoculated s.c. on the first and third week.

Radioimmunoassay Procedure

The wells of 96 well V-bottom polystyrene plates (Dynatech Laboratories, Alexandria, VA) were coated with peptide by diluting the peptides to 25 ug/ml in distilled water and evaporating 50 ul in each well by incubating the plates overnight at 37°C. Wells were coated with reovirus type 1 or type 3 by diluting stock solutions of virus to 4.8 x 10^{11} particles/ml in 0.1 M $NaHCO_3$ pH 9.5, dispensing 25 ul per well and incubation overnight at 4°C (London, S.D., Rubin, D.H., and Cebra, J.J., "Gut Mucosal Immunization with Reovirus Serotype I/L Stimulates Viral Specific Cytotoxic T Cell Precursors as Well as IgA Memory Cells in Peyer's Patches", 1987). Following overnight incubation, peptide or virus coated wells were washed 3 times with PBS and blocked with 200 ul/well of 1% gelatin in PBS with 0.1% NaN_3 by incubation for 2 hours at 37°C. The wells were decanted, washed 3 times in PBS, and mouse serum or purified monoclonal antibody was added, 50 ul/well, diluted in PBS containing 0.5% gelatin and 0.1% NaN_3. Following a 3 h ur incubation at 37°C, the wells were decanted, washed 3 times in PBS, and radioiodinated goat anti-mouse Kappa diluted in PBS 0.1% NaN_3 with 1 mg/ml chicken

gamma globulin was added, 100 ul = 48,000 counts per minute (CPM) per well. The plates were incubated overnight at 4°C, decanted, washed ten times in tap water and dried under a heat lamp. Wells were then cut out using a hot wire and counted in a gamma counter. The CPM determined on blank wells not coated with antigen is subtracted from CPM values determined on antigen coated wells in all cases.

Fluorescence Activated Cell Sorter (FACS) Analysis

R1.1 cells (99% viability to trypan blue dye exclusion) were centrifuged and washed twice in PBS 0.1% NaN_3 with 1% bovine serum albumin (FACS media). Cells were resuspended at 10^7/ml either in FACS media alone or FACS media containing peptide-BSA conjugates at 200 ug/ml. The cells were incubated on ice for 45 minutes prior to addition of monoclonal antibodies from 0.5 mg/ml stock solutions to 100 ul aliquots to the final concentrations noted. Following an additional 30 minute incubation, 500 ul of FACS medium was added to each sample, the cells were centrifuged, washed once in 500 ul FACS media, resuspended in 10 ul FACS containing a 1:200 dilution of fluoresceinated goat anti-mouse Fab (Southern Biotechnology Associates) and incubated for 30 minutes on ice. 500 ul of FACS media was added, the cells were centrifuged and washed in 500 ul FACS media, resuspended in 200 ul FACS media and analyzed at the University of Pennsylvania fluorescence activated cell sorter.

Neutralization of Virus Infectivity

The titer of neutralizing antibodies in serum sample were determined in the following manner: (i) Micro-neutralization: L-cells ($5x10^4$ per well) were incubated in 96 well dishes overnight at 37°C. Reovirus type 1/Lang (1/L) and type 3/D were serially diluted and incubated for 1 hour with the L-cells at 37°C. An additional 75 ul of MEM supplemented with 5% fetal bovine serum, 1% glutamine was placed in each well. At 3 days following incubation at 37°, the media containing virus was removed and the cells were stained with Gentian Violet (Gentian Violet 3.4 g/1, ammonium oxalate 8 g/1). The titer of virus used for neutralization

was 4 fold in excess of that quantity of virus that was lytic for the L-cell monolayer. Reovirus type 1/L or 3/D at the appropriate concentration was incubated with an equal volume of mouse serum for 1 hr at 25°C om 96 well plates. The virus-serum mixture was then transferred to L-cell monolayers as above. The titer of antibody was determined as the amount which preserved 70% of the monolayer as determined by visual inspection. (ii) Virus plaque reduction: 100 pfu of reovirus type 1/L incubated for 1 hour with L-cells ($7x10^5$ cells per well) in 12 well Costar plates. The titer of virus in each well was then determined as previously described (Rubin, D.H., Kornstein, M.J., and Anderson, A.D., Reovirus Serotype 1 Intestinal Infection: A Novel Replicative Cycle with Ileal Disease", J. Virol., 53:391-398, 1985).

Synthesis of Peptides:

Peptides were synthesized using a model 430A Applied Biosystems Peptide Synthesizer (Applied Biosystems, Inc., Foster City, CA. Deprotection and release of the peptide from the solid phase support matrix were accomplished by treating the protected peptide on the resin with anhydrous HF containing 10% aniscle or 10% thioanisole for 1 to 2 hr at 0 degrees C. The peptides were then extracted with either ethyl acetate or diethylether and then dissolved in 10% aqueous acetic acid and filtered to remove the resin. After lyophilization, the composition and purity of the peptides were determined with both amino acid analysis and reverse phase high performance liquid chromatography. This procedure was used for the synthesis of all peptides, including V_L and the variant peptides of V_L.

Conjugation of Peptides to Chicken Serum Albumin (CSA)

Prior to conjugating the peptides to CSA, the CSA was first derivatized with a nucleophilic spacer consisting of adipic dihydrazide, as described by Schneerson, et al., "Preparation, Characterization and Immunogenicity of Haemophilus Influenzae Type b Polysaccharide Protein Conjugates", J. Exp. Med., 152:361, (1980). 30 Mg of the adipic dihydrazide-derivatized-CSA (CSA-ADH) in 5 ml 0.1M

sodium bicarbonate was reacted for 15 min at room temperature with 7 mg m-maleimidobenzoylsulfosuccinimide ester (Pierce). To this reaction mixture was then added 50 mg peptide and the couples reaction was allows to proceed at 25 degrees C for 2 hr. Following dialysis against 0.1M ammonium bicarbonate and lyophilization, the CSA-ADH-peptide conjugates were obtained as dry white powders.

Determination of Shared Peptide Sequence

Prior work has shown that a monoclonal antibody denoted 87.92.6 raised against monoclonal neutralizing anti-reovirus antibody 9BG5 mimics the intact virus by binding to cell-surface receptors specific for type 3 reovirus Noseworthy, J.H., Fields, B.N., Dichter, M.A., Sobotka, C., Pizer, E., Perry, L.L., Nepon, J.T., and Greene, M.I., "Cell Receptors for the Mammalian Reovirus. I. Syngeneic Monoclonal Anti-Idiotypic Antibody Identifies a Cell Surface Receptor for Reovirus", J. Immunol., 131:2533-2538, 1983; Kauffman, R.S., etal, 1983 supra; Co, M.S., Gaulton, G.N., Fields, B.N., and Greene, M.I., "Isolation and Biochemical Characterization of the Mammalian Reovirus Type 3 Cell-Surface Receptor", Proc. Natl. Acad, Sci. USA, 82:1494-1498, 1985. 87.92.6 competes with reovirus type 3 for binding to specific cellular receptors thereby mimicking the viral cell attachment protein sigma 1 (the viral hemagglutinin) in its binding domain. This domain is also implicated in the neutralizing antibody response (Burstin, S.J., et al, 1982 supra; Spriggs, D.R., Kaye, K., and Fields, B.N., "Topological Analysis of the Reovirus Type 3 Hemagglutinin", Virology, 127: 220-224, 1983). This implies that 87.92.6 mimics the epitope on the hemagglutinin that interacts with the cellular receptor for reovirus. The nucleic acid sequences of the heavy and light chain variable regions (V_H and V_L respectively) of 87.92.6 have recently been determined (Bruck, C., Co, M.S., Slaoui, M., Gaulton, G.N., Smith, T., Fields, B.H., Mullins, J.I., and Greene, M.I., "Nucleic Acid Sequence of an Internal Image-Bearing Monoclonal Anti-Idiotype and its Comparison to the Sequence of the External Antigen", Proc. Natl. Acad. Sci.

USA, 83:6578-6582, 1986), and the sequences have been compared to that of the reovirus type 3 sigma 1 protein (Bassel-Duby, R., Jayasuriya, A., Chatterjee, D., Sonenberg, N., Maizel, J.V., Jr., and Fields, B.N., "Sequence of Reovirus Hemagglutinin Predicts a Coiled-Coil Structure", Nature, 315:421-423, 1985). In accordance with the methods of the present invention, shared sequence portions of the antigen and anti-idiotype have been identified. More particularly, a 16 amino acid sequence in the reovirus type 3 sigma 1 protein encompassing amino acids 317 and 332 has been identified as having amino acid sequence similarity to a combined sequence encompassing the second complementarity determining regions (CDR IIs) of the 87.92.6 heavy and light chain variable regions (V_H and V_L respectively). Specifically amino acids 43-51 of the V_H share sequence similarity with amino acids 317-324 of sigma 1 and amino acids 46-55 of the V_L share homology with amino acids 323-332 of sigma 1 (Bruck, C., et al, 1986, supra).

In accordance with the methods of the present invention peptides corresponding to amino acids 317-332 of the sigma 1 protein 43-50 of the V_H sequence and 39-55 of the V_L sequence have been synthesized. As demonstrated hereinafter, immunization of Balb/c mice with these peptides results in neutralizing anti-reovirus type 3 antibodies and specific cell-mediated immunity to reovirus. This establishes that the sequence homology between the sigma 1 cell attachment protein and the anti-receptor antibody predicts the neutralizing epitope on the reovirus hemagglutinin, sigma 1. This approach allows the rapid delineation of neutralizing epitopes on pathogens and the development of peptide vaccines that elicit a neutralizing response.

Binding of Neutralizing Monoclonal Antibody 9BG5 to Peptides

The monoclonal anti-receptor antibody 87.92.6 binds to both the reovirus type 3 receptor and the neutralizing antibody 9BG5 (Kauffman, R.S., et al, 1983, supra). Applicants predicted that the peptides derived from the areas of homology between 87.92.6 and the type 3 reovirus sigma 1

protein (Bruck, C., et al, 1986 supra) would have similar properties. The peptides synthesized to test this hypothesis are shown in Table 1.

Table 1

Synthetic Peptides Comprising the Area of Homology Between 87.92.6 and the Reovirus Type 3 Hemagglutinin

V_H	Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly
Reo	Gln Ser Met --- Trp Ile Gly Ile Val Ser Tyr Ser Gly Ser Gly Leu Asn
V_L	Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln
Control Peptide	Lys Ser Gly Asn Ala Ser Thr Pro Gln Gln Leu Gln Asn Leu Thr Leu Asp Ile Gln Arg

The peptides used in this study were synthesized by solid-phase methods as noted above. The sequences are shown aligned with maximum homology. The amino acids marked with a closed circle are identical and those marked with an open circle are of the same class. It will be noted that the tested peptides contain anti-idiotypic antibody residues in addition to the shared peptide sequence.

The reo peptide corresponds to amino acids 317-332 in the type 3 viral hemagglutinin. Computer modeling predicts this area to be predominantly a beta-sheet configuration and to include a beta-turn. The V_L peptide represents amino acids 39-55 of the light chain variable region of 87.92.6, and includes the second complementarity determining region (CDR II). Modeling predicts this area also to be a predominant beta-sheet and to include a beta-turn. The V_H peptide comprises amino acids 43-56 of the heavy chain variable region of 87.92.6 including CDR II of the heavy chain. The control peptide, unrelated to this system, is also shown.

Based on these similarities in primary and secondary structures, it was predicted that the reo and V_L peptides should be recognized by anti-reovirus type 3 neutralizing monoclonal antibody 9BG5. In Figur 1 we show the results of a radioimmunoassay determining the binding of purified monoclonal antibody 9BG5 to the wells of microtiter plates

coated with the peptides. To control for non-specific binding to the polystyrene wells, counts per minute (CPM) determined on blank wells not coated with peptide is subtracted from CPM values determined on peptide coated wells. In addition, since these peptides may also cause non-specific adherence of immunoglobulin molecules, we determined the specific binding of the class-matched irrelevant monoclonal antibody UPC-10 to peptide coated wells and subtracted this value from those determined for 9BG5. No significant binding was seen to the control peptide used in this study. Similarly, binding to the V_H peptide only achieved background levels indicating that this epitope is not recognized by 9BG5. There was a small amount of binding to the V_L peptide, which has strong homology in its carboxy terminal sequence to the reo peptide carboxy terminal. Although slight, this finding was reproducible on subsequent assays. Strong reproducible binding to the reo peptide by 9BG5 was evident. Since 9BG5 is a neutralizing antibody, this datum implies that the reo peptide contains the neutralizing epitope recognized by 9BG5. The binding to the V_L peptide indicates that the area of sequence homology between these peptides (amino acids 323-332 of the sigma 1 protein) is involved in the neutralizing epitope.

Binding of V_L Peptide to the Reovirus Receptor

Prior work indicated that the neutralizing epitope recognized by 9BG5 is involved in binding to the type 3 reovirus receptor (Kauffman, R.S., et al, 1983, supra; Noseworthy, J.H., et al, 1983, supra; Spriggs, D.R., et al, 1983, supra). This led us to speculate that the V_L peptide might also interact with the viral receptor. To test this hypothesis we coupled the V_H and V_L peptides to BSA by incubating peptides and BSA in 0.1% glutaraldehyde followed by dialysis against PBS and used these preparations to determine if we could specifically block 87.92.6 binding to the type 3 reovirus receptor on R1.1 cells. As shown in Figure 2, pre-incubation of R1.1 cells with V_L-BSA blocked the binding of 87.92.6 indicating interaction of V_L-BSA with the reovirus receptor. This blocking effect is specific as

pre-incubation of R1.1 cells with V_L-BSA had no effect on the binding of HO 13.4, and isotype matched control monoclonal antibody that binds to the Thy 1.2 molecule on the R1.1 cell surface (Figure 3). These observations were consistently reproducible on multiple experiments. An additional control is shown in Figure 3 where it is demonstrated that V_H-BSA has no inhibitory effect on 87.92.6 binding when used at the same concentrations as V_L-BSA. These data indicate a direct interaction of the V_L peptide with the reovirus type 3 receptor and imply that residues 46-55 of the 87.92.6 V_L chain and 323-332 of the type 3 sigma 1 protein directly interact with the reovirus type 3 receptor.

Binding of Reovirus type 3 Inhibits Host Cell DNA Synthesis Upon Receptor Perturbation

Reovirus type 3 inhibits host cell DNA synthesis upon receptor perturbation. This effect is not due to infection of cells as replication defective reovirus type 3 particles retain this property. L cells were cultured at 5 X 10^4 cells per well of 96 well microtiter plates in 100 ul media for 24 hours. Reovirus type 3 particles (A) were added and incubated for an additional 24 hours prior to the addition of tritiated thymidine. Purified monoclonal antibodies 87.92.6 or HO 22.1 (B) were added for 1 hour at 37°C, then the culture media removed and replaced with 100 ul fresh media for 24 hours, prior to the addition of tritiated thymidine. The cells were incubated for an additional 4-6 hours and counts per minute (CPM) incorporated were determined. Figure 15 shows this effect of reovirus type 3 upon murine fibroblasts. Murine fibroblasts (which posses specific receptors for reovirus type 3) (L cells), were incubated with reovirus type 3, or left untreated (8A). Twenty-four hours later the DNA synthetic level was measured. Reovirus type 3 markedly inhibited DNA syntheses by these cells. 87.92.6 has a similar effect on these cells, as shown in Figure 15. In this experiment, L cells were grown adherent and exposed to antibody for one hour, at which point the antibody was removed, and the cells cultured for an additional 24 hours

prior to determination of the DNA synthesis while a control antibody (HO22.1) had no effect. 87.92.6 similarly inhibits DNA synthesis by fibroblasts, neuronal cells and lymphocytes.

Binding of Dimeric Peptides to Reovirus Type 3 Receptors

It was hypothesized that V_L peptide may exhibit biologic effects similar to those exhibited by reovirus type 3 and 87.92.6. 87.92.6 is effective only as a native antibody while monomeric Fab fragments have no effect. V_L peptide was synthesized with an additional amino terminal cysteine residue (V_LSH) to form a dimeric peptide. VL_SH peptide was dimerized by stirring a 5 mg/ml solution in 0.1 M ammonium bicarbonate overnight at 23C exposed to air. The peptides were then lyophilized. Dimerization was confirmed by Ellman determination according the the procedure of Ellman, G.L. Arch. Biochem. Biophys. 74: 443 (1958), which revealed less than 5% free sulfhydryl groups. L Cells were suspended at 10^6 cells per ml in DMED 10% FBS and 50 ul added to each well of 96 well microtiter plates. Following 24 hours of culture, peptides were added to the concentrations noted, and the cells cultured for an additional 24 hours. Tritiated thymidine was added for an additional 4-6 hours, and counts per minute (CPM) incorpoated was determined. Per cent inhibition was determined by the formula:

$$\left[1 - \frac{\text{(CPM without additive) - (CPM with additive}}{\text{CPM without additive}} \right] \times 100$$

The peptides utilized were:

V_L: Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln

V_LSH: Cys-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln

Control: Cys-Tyr-Thr-Tyr-Pro-Lys-Glu-Asp-Thr-Ala-Asn-Asn-Arg

As shown in Figure 16, marked inhibition of DNA synthesis was observed when L cells were treated with V_LSH. V_L peptide monomers (without the added cysteine residue) had no effect on L cell proliferation. Several control peptides utilized also had no effect in these assays (Figure 16). This

indicates that aggregation of the reovirus type 3 receptor on L cells is essential for the inhibition of DNA synthesis by these peptides.

Down-Modulation of Reovirus Type 3 Receptor By Peptide Dimers

Aggregation of the reovirus type 3 receptor on some cells by 87.92.6 leads to disappearance of that receptor from the cell surface. It was hypothesized that V_LSH peptide might similarly down-modulate this receptor. For these experiments murine thymoma (R1.1) cells, which have well characterized reovirus type 3 receptors were used. The effect of peptides on the level of expression of both the reovirus type 3 receptor (recognized by 87.92.6) and Thy 1.2 molecules (recognized by HO 13.4), as determined by flow cytometry was studied according to the following method. R1.1 cells were cultured with peptides at the concentration noted (A), left untreated (B), or treated with 500 ug/ml peptide (C,D) for 1 hour at 37°C. The cells were centrifuged and washed 3 times in 1% bovine serum albumin in phosphate buffered saline with 0.1% sodium azide (FACS media). Monoclonal antibodies 87.92.6 (100 ul of affinity purified antibody) was added for 30 minutes on ice. The cells were washed and 100 ul of a 1:100 dilution of fluorceinated goat anti-mouse Ig (Southern Biotechnology Associates, Birmingham, AL) was added for 30 minutes. The cells were washed and fluorescence intensity analyzed by flow cytometry. Mean channel fluorescence was compared for cells incubated in the presence or absence of primary antibody to give mean chanel fluorescence (Figure 30). Cells were stained with HO 13.4 (Figure 16, left panels in A, B, C, and D) which binds Thy 1.2 molecules, or with 87.92.6 (Figure 16, right panels in A, B, C, and D) which binds the reovirus type 3 receptor. Cells were treated with V_H peptide (Figure 17 and Figure 19) or VLSH peptide (Figure 18 and Figure 20).

The V_H peptide sequence:

V_H: Cys-Gln-Gly-Leu-Glu-Gln-Ile-Gly-Arg-Ile-Pro-Ala-Asn-Gly

The other peptides are those described above for Figure 16. As shown in Figure 20, V_LSH peptide specifically down-modulates the reovirus type 3 receptor in a dose-dependent manner, but does not effect the expression of Thy 1.2 molecules on these cells. This down-modulation is a direct biologic effect of V_LSH peptide and not due to other factors in the experimental design. The control peptide used (V_H peptide) does not effect the level of expression of the reovirus type 3 receptor, or of Thy 1.2 molecules, on these cells. V_H peptide was derived from the 87.92.6 heavy chain CDR II and does not specifically interact with the reovirus type 3 receptor. It has been demonstrated previously that V_L peptide in this form does not compete with 87.92.6 for binding to these cells, although other forms of V_L peptide are able to inhibit 87.92.6 binding. In addition, in the studies described in Figure 20, the cells were washed thoroughly to remove free V_LSH peptides prior to flow cytometry. Collectively these data indicate that competition for binding to the reovirus type 3 receptor is not responsible for the decreased staining with 87.92.6. We conclude that down-modulation of the reovirus type 3 receptor accounts for this phenomenon.

Receptor down-modulation is dependent on aggregation of the receptor, as demonstrated in Figure 21. Data from three experiments comparing the effect of V_L peptide monomers and V_LSH peptide is shown. R1.1 cells were treated as described above with peptides (100 ug/ml) or 87.92.6 (a 1:1 dilution of ascites), and analyzed for expression of the reovirus type 3 receptor (87.92.6) or Thy 1.2 molecules (HO 13.4). Per cent decrease in mean channel fluorescence is calculated as follows: The mean channel fluorescence of peptide or antibody treated cells is subtracted from that of untreated cells, this divided by the mean channel fluorescence of untreated cells; the resultant value is subtracted for 1 and multiplied by 100. For peptide treated cells, mean

channel fluorescence is determined on peptide treated cells in the presence or absence of primary antibody. For antibody treated cells, mean channel fluorescence is determined by the mean channel number of antibody treated cells in the presence of primary antibody minus the mean channel number of untreated cells in the absence of primary antibody. Cells treated with antibody and then alayzed without primary antibody staining had an increase in mean channel number when compared with untreated cells. The mean ± standard deviation from 3 experiments is shown for peptide treated cells. The peptides used in these experiments were

V_L: Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln

V_LSH: Cys-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln

Control: Cys-Thr-Tyr-Arg-Pro-Lys-Glu-Asp-Thr-Ala-Asn-Asn-Arg

V_L peptide monomers had no effect on reovirus type 3 receptor expressions. V_LSH peptide specifically down-modulated the expression of the reovirus type 3 receptor without effecting the expression of Thy 1.2 molecules. The effect of V_LSH peptide was similar to that of 87.92.6 (Figure 21). The results indicate the specificity of the effect of V_LSH peptide on the reovirus type 3 receptor and confirm that receptor aggregation plays a role in the induction of these effects.

Role of Specific Residues Of V_L Peptide Involved In The Interaction Of V_L Peptide With The Reovirus Type 3 Receptor

Once the shared regions were defined, variant peptides with substitutions at several positions in the putative binding domain of V_L peptide were synthesized to study the effect of these forms of the peptide on cellular physiology. These studies indicate that hydroxyl groups from positions 11 (Tyr), 12 (Ser), 14 (Ser) and 15 (Thr) may be involved in directly interacting with the reovirus type 3 receptor. This is the region of greatest shared identity of amino acids between the V_L peptide and the reo peptide. See

Table 1. The variant peptides had amino acid substitutions at positions 11-16, the region of the V_L peptide believed to be the binding domain. To study the effect of these forms of peptide on cellular physiology, we have utilized lectin induced mitogenesis to provide a system wherein we can induce both receptor perturbation (by the peptides) and aggregation (by the lectin).

Peptide Inhibition of Lymphocyte Proliferation

Reovirus type 3 and anti-reovirus type 3 receptor antibodies have both been demonstrated to inhibit concanavalin A (con A) induced lymphocyte proliferation (Nepom, J.T. et al., Immunol. Res. 1: 255 (1982), Sharpe, A.H. and B.N. Fileds, J. Virol. 38: 389 (1983), Fontana,A. and H.L. Weiner, J. Immunol. 125: 2660 (1980)). The effects of these peptides and variant peptides on lymphocyte proliferation both in the presence and in the absence of con A were determined using the following method. C3H female mouse spleenocytes were prepared as a single cell suspension, and cultured with peptides at the concentrations noted in absence (A) or in the presence (B) of concanavalin A (con A) at 2.5 ug/ul. 72 hours later tritiated thymidine was added, the cells were harvested 18 hours later and CPM incorporated determined. Per cent inhibition was calculated as for Figure 16. The peptides utilized are those described for Figure 16. In the absence of con A, V_LSH peptide markedly inhibited spontaneous lymphocyte proliferation, while V_L peptide had no significant effect (see Figure 22). However, in the presence of con A, V_LSH peptide and V_L peptide had similar effects in inhibiting lymphocyte proliferation (see Figure 23).

As shown in Figure 24, when variant peptides were utilized lacking hydroxyl groups from positions 12 and 15 (V_LA12 and V_LA15 respectively), the inhibition of con A induced lymphocyte proliferation was attenuated (Figure 24).

Lymphocyte proliferation was determined as described above for Figure 22. The peptides utilized were:

V_L - Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln,

V_LF11- Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Phe-Ser-Gly-Ser-Thr-Leu-Gln,

V_LA12- Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ala-Gly-Ser-Thr-Leu-Gln,

V_LA13- Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Ala-Ser-Thr-Leu-Gln, and

V_LA15- Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Ala-Leu-Gln

This indicates that these amino acid residues are involved in interactions critical to receptor perturbation, leading to inhibition of proliferation. The hydroxyl groups of positions 11 (Tyr) and 14 (Ser) appeared to have less of an effect on this cellular activity (Figure 25).

A peptide with a (Gly-Ala substitution at position 13 in the putative binding domain of V_L peptide (V_LA13) was also tested. In contrast to the other substitutions described, V_LA13 had an increased effect on the inhibition of Con A induced lymphocyte proliferation at some of the concentrations used (Figure 25). This V_LA13 peptide also has increased binding to monoclonal antibody 9BG5, which may mimic the reovirus type 3 receptor on these cells. These studies indicate that modification of the V_L peptide can identify specific residues required for receptor perturbation, and lead to the development of variant peptides with both increased and decreased biologic activity.

<u>Competitive Binding of 9BG5 to 87.92.6 In The Presence Of Peptides</u>

Polystyrene wells were coated with purified 87.92.6 or control IgM, K antibody HO22.1 by incubation of purified antibody (purified on a goat anti-mouse IgM column), diluted in 0.1% $NaHCO_3$ pH 9.5 to 1 ug/ml with 50 ul/well, overnight at 4C. The wells were washed, blocked with 2% BSA in PBS with

0.1% NaN_3, washed again and a mixture of radioiodinated 9BG5 and peptides (at the concentrations noted in Figure 26) were added for one hour at 37C. The wells were washed and counted. In all cases, specific CPM bound was determined by subtracting CPM bound to blank wells coated with BSA from CPM bound for 87.92.6 coated wells. As shown in Figure 26, binding of ^{125}I-9BG5 to wells coated with irrelevant mouse IgM, K antibody HO22.1 was similar to binding to blank wells. Per cent inhibition was determined by subtracting specific CPM bound in the presence of inhibitor from specific CPM bound in the absence of inhibitor, dividing this by CPM bound in the absence of inhibitor, and multiplying the result by 100. The ± SEM of values from two experiments is shown in Figure 26.

V_L Peptide Inhibits Binding Of Reovirus Type 3 Particles to 9BG5.

The wells of mocrotiter plates were coated with neutralizing anti-reovirus type 3 monoclonal antibody 9BG5 or irrelevant class matched monoclonal A11 by adsorption to staphylococcal protein A (SPA). SPA (sigma Chemical Co., St. Louis, MO) was diluted to 5 ug/ml in 0.1 M $NaHCO_3$ pH 9.6 and 50 ul/well dispensed into 96 well polystyrene plates. Following overnight incubation at 4C, the wells were decanted, washed 3 times in PBS, and blocked with 2% BSA in PBS with 0.1% NaN_3 for one hour at 37C. The wells were decanted, washed 3 times in PBS and monoclonal antibody 9BG5 diluted to 10 ug/ml in 1% BSA in PBS with 0.1% NaN_3 was added (50 ul/well) for 1-3 hours at 37C. Prior studies indicated that these amounts of SPA and monoclonal murine IgG2a antibodies gave maximal adsorption of antibody on the wells. The wells were decanted and washed 3 times in PBS. Competitors were added at the concentrations noted (100 ul/well) diluted in 0.5% BSA in 5 mM phosphate buffer with 0.45% NaCl and preincubated for 45-60 minutes at 23C. Control experiments indicated that these peptides had no effect on monoclonal antibody binding to the wells. Following preincubation with inhibitors, radioiodinated reovirus type 3 particles diluted in 1% BSA in PBS with 0.1% NaN_3 were added (5-10 x 10^5 CPM per

well), and the incubation continued for 45 minutes. Wells were decanted, washed 8-10 times with PBS and the CPM bound determined. V_L peptide inhibits binding of reovirus type 3 particles to 9BG5. As shown in Figure 27, 6,700 CPM were bound to 9BG5 coated wells and 500 CPM were bound to control (A11) coated wells in the abscence of inhibitors. The mean ± standard deviation of binding inhibition (Determined as noted for Figure 26) of replicate wells is shown. Control peptide B was used in this study. The competitor peptides in Figure 27-29 are those described herein. Competitor peptide V_LA6 is identical to V_L except that alanine is substituted for asparagine at position 6. The competitor peptides inhibited binding of reovirus type 3 particles to 9BG5.

V_L Peptide Inhibits Binding of Reovirus Type 3 and variant K to L Cells

L cells were suspended at 10^6/ml in 1% BSA in PBS with 0.1% NaN_3, and 50 ul ($5x10^4$) cells) added to each well of a 96-well microtiter plate, and preincubated with inhibitors at the concentrations noted for 45-60 minutes at 23C. Equivalent input CPM of radioiodinated reovirus type 3, type 1 or variant K particles were added in 50 ul (700,000 to 1,250,000 CPM/well) and incubated for 45 minutes. The cells were washed 3 times in 1% BSA in PBS with 0.1% NaN_3 and specific CPM bound determined, as noted in Figure 26. As shown in Figures 30 and 31, V_L peptide inhibits binding of reovirus type 3 and variant K to L cells. The mean ± S.D. percent inhibition of binding from replicate wells is shown versus the final concentration of competitor. As shown in Figures 32 and 33, V_L variant peptides also inhibit binding of reovirus type 3 to murine L cells.

Immunization with Peptides Induces Reovirus-Binding Antibodies

The V_L and reo peptides contain the epitope involved in the interaction between type 3 reovirus and its specific cellular receptor. Immunization of mice with these peptides was done to determine whether they would induce antibodies capable of interacting with reovirus type 3 and blocking infection. Groups of Balb/c mice were immunized with these

synthetic peptides as noted in the experimental procedures section. Groups of 4 mice received either control peptide in adjuvant, V_L peptide coupled to chicken serum albumin (V_L-CSA) in adjuvant, V_H and V_L peptide coupled to CSA (V_H + V_L-CSA) in adjuvant, reo peptide in adjuvant or reo peptide without adjuvant. As a positive control an additional group of mice was injected with reovirus type 3. As indicated below, pre-immune serum from these mice disclosed no reovirus neutralizing antibodies indicating no prior exposure to virus.

Radioimmunoassay indicated a strong response to the immunizing antigen in all cases (data not shown). Binding of immune serum (day 60) to reovirus type 1 and type 3 is shown in Figures 4-7. Specific binding was determined by subtracted CPM bound on a blank plate from CPM bound on a virus coated plate. As a further control, specific binding of normal mouse serum to virus coated plates was also subtracted. To simplify interpretation, specific binding is shown for four groups of animals: Those immunized with the control peptide, V_L-CSA, V_H+V_L-CSA (all with adjuvant), and reo peptide without adjuvant. Mice immunized with reo peptide plus adjuvant made a response similar to those immunized with V_L-CSA or V_H+V_L-CSA plus adjuvant. Mice immunized with type 3 reovirus made a strong response to type 3 virus (specific CPM at a 10^{-3} dilution of serum of $10,428 \pm 807$) with significant cross-reactivity with type 1 virus (specific CPM at a 10^{-3} dilution of $6,976 \pm 915$). As shown in Figures 4-7, serum from mice immunized with control peptide bound poorly to type 1 or type 3 virus coated plates at any of the serum dilutions used. In contrast, significant binding of immune serum to type 1 and type 3 virus coated plates is demonstrated from mice immunized with V_L-CSA, V_H + V_L-CSA or reo peptide. As was expected, binding to type 3 virus was significantly higher than binding to type 1 virus, although some cross-reactivity is seen. The binding of type 1 virus was likely to have been due to some areas of primary sequence homology between the peptides used here and the type 1 sigma 1a protein (Manemitsu, S.M., Atwater, J.A., and Samuel, C.E., "Biosynthesis of

Reovirus-Specified Polypeptides. Molecular cDNA Cloning and Nucleotide Sequence of the Reovirus Serotype 1 Long Strain Bicistronic S1mRNA Which Encodes the Minor Capsid Polypeptide /a and the Nonstructural Polypeptide 16NS", Biochem. Biophys. Res. Commun., 140:501-510, 1986).

These results indicate that priming mice with peptides modeled from the putative neutralizing epitope of type 3 reovirus or the corresponding epitope from the anti-receptor monoclonal antibody induces reovirus binding antibodies.

Neutralization of Viral Infectivity by Immune Serum from Peptide Immunized Mice

Serum from peptide immune animals was assayed at three time points to evaluate its effects on viral infectivity of L-cells. Two assays were used to detect neutralization of infectivity. One was a direct cytotoxicity assay measuring the effect of serum on viral lysis of L-cells grown adherent to the wells of 96-well microtiter plates by vital staining, and the other was by measuring inhibition of plaque formation by serum, with virus and L-cells in soft agar. Results from the direct cytotoxicity assay are shown in Figures 8 and 9. Pre-immune serum from all of the animals used was assayed and no significant effect on type 1 or type 3 viral lysis of L-cells was demonstrated. As a positive control, neutralization of L-cell lysis by reovirus was demonstrated by serum from mice immunized with reovirus type 3. This serum produced potent inhibition of lysis by both type 3 and type 1 virus, although a preferential effect on type 3 viral lysis was noted, with neutralization titers of 1:512 for type 3 virus on days 20 and 60, and titers of 1:342 and 1:256 for type 1 virus on days 20 and 50 respectively. Serum from control peptide immunized animals had no effect on L-cell lysis by reovirus type 1 or type 3. Serum from mice immunized with V_L-CSA, V_H + V_L-CSA, or reo peptide with or without adjuvant specifically neutralized L-cell lysis by reovirus type 3 but not type 1 (Figure 8 versus 9). As results were similar for serum from animals immunized with reo peptide in

the presence or absence of adjuvant, results only from the latter group is shown. This effect was also seen when serum from these mice was assayed for inhibition of plaque formation. In Figures 10 and 11, the reciprocal serum titer producing 50% of greater plaque inhibition is shown for type 1 and type 3 virus from the groups immunized with V_L-CSA, V_E + V_L-CSA or reopeptide without adjuvant. Again, specific inhibition of plaque formation by type 3 but not type 1 virus is seen. Since peptide-immune serum specifically inhibits type 3 but not type 1 viral infectivity, these peptides define the neutralizing epitope present on reovirus type 3.

Elicitation of Delayed-Type Hypersensitivity (DTH) to Reovirus Type by Immunization with Peptides

Prior studies have demonstrated that the specificity of DTH responses to reovirus infection involved the sigma 1 polypeptide (Weiss, H.L., Greene, M.I., and Fields, B.N., "Delayed Type Hypersensitivity to Mice Infected with Reovirus. Identification of Host and Viral Gene Products Responsible for the Immune Response", J. Immunol., 125:278-282, 1980). It was therefore determined if immunization of mice with these peptides would elicit DTH responses to intact reovirus. As shown in Figures 13 and 14, significant DTH responses to reovirus type 3 were induced by immunization with V_L peptide. This response was type specific as these animals did not demonstrate significant DTH responses to reovirus type 1. Use of reassortant viruses maps the response to the sigma 1 protein. In addition, priming animals with type 3 virus results in significant DTH to the V_L peptide. We have also recently demonstrated a type specific proliferative response to reovirus type 3 in spleen cells from mice immunized with reo peptide. These data indicate that V_L and reo peptide define an important epitope involved in T cell-mediated immunity to reovirus type 3.

Discussion

It has thus been demonstrated that synthetic peptides, defined by areas of sequence homology between the

reovirus type 3 sigma 1 polypeptide and a monoclonal anti-receptor antibody 87.92.6, define the epitope on the virus and on the antibody involved in interacting with neutralizing antibody 9BG5, elicit neutralizing antibodies and induce T-cell mediated immunity. In addition, it has been demonstrated that one of these peptides, V_L, competes with binding of 87.92.6 to the reovirus type 3 receptor on R1.1 cells. Since 87.92.6 competes with reovirus type 3 binding to R1.1 cells (Kauffman, R.S., et al, 1983, supra), it is hypothesized that this epitope in the virus is involved in directly interacting with the type 3 reovirus receptor. This is confirmed by the ability of V_L peptide to inhibit binding of reovirus type 3 to cells. Binding of reovirus type 1 (which utilizes a distinct receptor) is not inhibited, indicating a specific interaction with the reovirus type 3 receptor. Since this epitope encompasses amino acids 317-332 of the sigma 1 polypeptide, this finding would seem at odds with other reports which have implicated amino acid 419 of the hemagglutinin in viral resistance to neutralizing antibodies (Bassel-Duby, R., Spriggs, D.R., Tyler, K.L., and Fields, B.N., "Identification of Attenuating Mutations on the Reovirus Type 3 S1 Double-Stranded RNA Segment With a Rapid Sequencing Technique", J. Virol., 60: 64-67, 1986), and in tissue tropism of the virus (Kaye, K.M., Sprigg, D.R., Bassel-Duby, R., Fields, B.N., and Tyler, K.L., "Genetic Basis for Altered Pathogenesis of an Immune-Selected Antigenic Variant of Reovirus Type 3 (Dearing)", J. Virol., 59: 90-97, 1986). In those studies, viruses were selected for by growth in the presence of neutralizing antibodies (Spriggs, D.R., and Fields, B.N., "Attenuated Reovirus Type 3 Strains Generated by Selection of Hemagglutinin Antigenic Variants", Nature (London), 297:68-70, 1982), and those resistant to neutralization by the antibodies had their amino acid sequence determined (Bassel-Duby, R., et al, 1986, supra). Several possibilities might account for the disparity in these results. It is possible that the mutations involving the amino acids 419 induce an allosteric effect on the

conformation of amino acids 317-332 which allows interaction with the viral receptor in the presence of the neutralizing antibodies. In this scenario, amino acids 317-332 would be directly involved in binding to the viral receptor and to neutralizing antibody. The mutation at amino acid 419 would induce an allosteric alteration in the confirmation of this region that would allow binding to the viral receptor in the presence of neutralizing antibody. Another possibility is that both regions are involved in binding the viral receptor. In this case both regions would be in close proximity in the tertiary structure of the sigma 1 polypeptide. This is possible as both are predicted to be in the "globular head" region of the hemagglutinin by computer modeling (Bassel-Duby, R., et al, 1985, supra). The mutation of 419 would strengthen the interaction of this area of the hemagglutinin with the receptor, thereby overcoming the blockage of receptor binding by the neutralizing antibodies binding to residues 317-332. While other possibilities exist, clarification of these issues awaits more detailed knowledge of the tertiary structure of the sigma 1 protein.

These studies have direct implications for vaccine development. It would be greatly desirable to be able to delineate the neutralizing epitopes present on microorganisms to aid in development of synthetic vaccines that would effectively protect individuals from infection, without the risks involved in the use of whole organisms. This would be particularly useful in situations where there is marked antigenic heterogeneity in the structure of a pathogen, but the binding site for specific cellular receptors is conserved. A variety of strategies can be and have been employed to determine sites involved in receptor-pathogen interactions including site-directed mutagenesis and immunization of animals with sequential peptides derived from the sequences of pathogen products (Elder, J.H., et al, 1987, supra). Site directed mutagenesis, while yielding specific information about sequence variations that lead to differences in biological effects, suffers from the disadvantage that

allosteric effects resulting from the sequence differences could account for the effects induced. In this situation, sequence variation in a region of a gene product may alter the biologic properties of a distant site and yield misleading information. Analysis of the effects of antibodies elicited by immunization with sequential peptides derived from pathogen products, while a definitive approach yielding specific information, is time-consuming and may require analysis of a large number of peptides before a neutralizing immune response is detected.

Significance

The above experiments thus demonstrate a method for producing a synthetic biologically active peptide comprising a sequence corresponding to a peptide sequence found in corresponding regions of both an antigen and in an anti-idiotypic antibody for that antigen. By demonstrating sequence homology between the sigma 1 cell attachment protein of reovirus type 3 and monoclonal anti-receptor antibody 87.92.6 the neutralizing epitope of reovirus type 3 was localized. These studies confirm that the epitope implicated is the one involved in viral binding to the cellular reovirus type 3 receptor and in the elicitation of neutralizing antibodies.

Once the shared region has been defined, other biologically active peptides can be prepared by modifiying this peptide sequence. These modifications are directed to the region believed to be involved in binding of the antigen to the receptor. Gly(13) and hydroxyl groups from positions 11(Tyr), 12(Ser), 14(Ser) and 15(Thr) are believed to be involved in directly interacting with the reovirus type 3 receptor. Peptide dimers comprising the shared peptide sequence also have biological activity. As the studies herein indicate, modification of the V_L peptide can lead to development of variant peptides with both increased and decreased biological activity. Peptide V_LA12 has reduced binding to neutralizing monoclonal antibody, reduced binding to the reovirus type 3 receptor and reduced biologic activity. Peptide V_LA15 has increased binding to neutralizing monoclonal

antibody, decreased binding to the reovirus type 3 receptor and decreased biological activity. Variant peptides such as V_LA12, if used as immunogens, might prevent an effective immune response. However, this might be clinically useful in some instances. V_L peptide itself, if used as an immunogen, might elicit an effective immune response, but direct effects of the V_L peptide on the retrovirus type 3 receptor might be deleterious to the host. In this case, a variant peptide such as V_LA15, which binds to nwutralizing antibodies, but has reduced biologic activity, might be ideal as an immunogen as it would elecit neutralizing antibodies but would not be expected to have significant direct effects on the retrovirus type 3 receptor and would not be expected to be deleterious to the host. The present approach of defining a shared peptide region of both an antigen and an anti-idiotypic antibody for that antigen and subsequently modifying this peptide to produce peptides having more or less biological activity is believed to be generally applicable to other receptor-ligand interactions.

The present approach further demonstrates a method of immunizing a host mammal against an infectious organism having a site which binds specifically to a receptor site on a host cell. This method allowed for the relatively rapid determination of the neutralizing epitope on reovirus type 3 and is believed to be generally applicable to other pathogens for which neutralizing immune responses can be demonstrated.

In the present instance, the reovirus type 3 is known to selectively bind to a structure similar to the mammalian beta-adrenergic receptor. If attachment of a pathogen to specific cellular receptors is important in the pathogenesis of infection by that pathogen, the approach outlined here should result in the ability to determine the oligopeptide epitope involved in the pathogen-receptor interaction. This should also be applicable to other receptor-ligand interactions in a more general sense, and in the case of polypeptide ligands, should allow the determination of the binding epitopes involved. It is believed

that this strategy will lead to the development of biologically active substances that will interact with specific receptors in predictable ways. Accordingly a method is disclosed which is useful for synthesizing biologically active compounds using pathogen gene products, such as the reovirus 3, which is known to bind to a cellular receptor of mammalian cells. Where, as with the mammalian reovirus type 3 receptor, the result of such selective binding is to affect the growth or other metabolic function of the subject cell, the subject method may be used for altering the growth of the mammalian cell by administering the synthetic peptide containing the subject shared peptide sequence or biologically active modification thereof for that purpose.

The present approach also provides an alternative route for the development and production of biologically active peptides. As shown in Figure 34, antibodies 15 specific for a receptor 11 of the antigen (or ligand) 5 also mimic the antigen 5 in the same way as an anti-idiotype antibody 9 of the antigen mimics the antigen 5. In pathway I, an antibody 7 contains an epitope designated generally 21 complementary to the neutralizing epitope designated generally 19 of antigen 5. This antibody 7 is then used to produce other antibodies, or anti-idiotype antibodies 9. These anti-idiotype antibodies 9 will have a region designated generally 23 mimicking the neutralizing epitope 19 of the antigen 5. In pathway II, the receptor 11 on cell surface 13 contains an epitope designated generally 25 complementary to the neutralizing epitope 19 of the antigen 5; the antibody specific for the receptor 15 will thus contain a region designated generally 27 mimicking the neutralizing epitope 19 of the antigen 5. The anti-receptor antibody 15 is the equivalent of anti-idiotype antibody 9, since both contain regions (23 and 27) mimicking the neutralizing epitope 19 of the antigen 5. Anti-receptor antibodies 15 can be used as an alternative, or in addition to, anti-idiotype antibodies 9 in the methods described herein to develop and produce

biologically active peptides 17 with properties of the antigen or ligand.

Because antigens such as viruses generally contain multiple antigenic epitopes, it may be necessary to screen the antibodies produced in response to the inoculation with the ligand, receptor or anti-ligand antibody to select antibodies having specificity for the neutralizing epitope of the antigen. Screening can be done by competitive assays that determine the antibody's ability to inhibit binding of the antigen to the receptor of the cell, those antibodies having a greater ability to inhibit binding of the antigen containing or mimicking the neutralizing epitope. Suitable screening methods include those described herein, and in It will be obvious to those skilled in the art that various changes to reagents may need to be made in the competitive assays when different antigen and receptor pairs are used.

As demonstrated herein, neutralizing antibody 9BG5, having a specificity for the antigen HA3 on the reo virus, was used to make anti-idiotype antibodies having anti-receptor activity. These anti-idiotype antibodies also bind to the reovirus type 3 receptor. The antibodies were screened to identify antibodies that competed or inhibited binding of the neutralizing antibody with the receptor which would indicate they contained epitopes that mimic HA3, the antigen. The variable region of one antibody having this activity was compared with the sequence of the antigen HA3 to determine regions of homology that define the interaction site of HA3 and the receptor.

Instead of using an anti-receptor antibody that was produced as an anti-idiotype antibody, the receptor itself is also suitable for producing antibodies that have epitopes mimicking the antigen. To produce antibodies by this route, the receptor, typically a protein or glycoprotein is isolated from cells having the receptor by standard techniques for isolating proteins, such as The purified receptor is then used to make antibodies, usually monoclonal antibodies, by conventional techniques. An animal such as a mouse is first

injected with the receptor, its spleen cells are removed and fused with myeloma cells to f rm hybrid ma cells, the latter are cloned in a serum-containing medium and the monoclonal antibodies are separated from the medium. The antibodies are then screened by neutralization assay, as described above, to select those antibodies which specifically bind to the receptor site at the neutralizing epitope.

The strategy of utilizing shared primary structure and molecular mimicry to define interacting oligopeptide epitopes thus should have a wide range of applications in the biological sciences to both define areas of specific interaction between molecules, and to aid in the development of substances with predictable biologic activity.

Those of ordinary skill in this art recognize that various modifications can be made in the compounds (peptides) of the present invention without departing from the scope hereof. For example, peptides of the same class (i.e., conservative substitution as described by Chu et al, "Conformational Parameters For Amino Acids in Helical, Beta Sheet, and Random Coil Regions Calculated from Proteins", Biochemistry, 13(2):211 (1974) (which is hereby incorporated by reference) may be substituted in the sequence shared between the antibody and antigen, provided the activity of the resulting peptide is not adversely affected. Similarly, it is contemplated that molecular modeling techniques will permit compounds of quite different primary and secondary structures to be substituted for the peptides of this invention, provided equivalent tertiary structures, as determined using the methods of this invention are employed. Additionally, other antibodies, such as other anti-receptor antibodies to the reovirus type 3 receptor or anti-idiotypic antibodies to neutralizing antibodies may also contact the receptor using CDR regions. Peptides derived from these regions having biologic activity similar to that described herein for V_L peptide are als within the scope of the invention.

CLAIMS

What is claimed:

1. A synthetic biologically active peptide comprising a peptide sequence corresponding to a peptide sequence found in corresponding regions of both an antigen and an anti-idiotypic antibody for said antigen or an antibody specific for a cellular receptor of said antigen.

2. A synthetic biologically active peptide dimer comprising first and second peptide sequences, each of which correspond to a peptide sequence found in corresponding regions of both an antigen and an anti-idiotypic antibody or an antibody specific for a cellular receptor of said antigen, said dimer being joined at one end.

3. The peptide dimer of claim 2 wherein said first and second peptide sequences are joined by a sulfhydryl bond.

4. A synthetic biologically active peptide of claim 1 or claim 2 wherein said peptide has the following amino acid sequence

X-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln,

X-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Phe-Ser-Gly-Ser-Thr-Leu-Gln,

X-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ala-Gly-Ser-Thr-Leu-Gln,

X-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Ala-Ser-Thr-Leu-Gln, or

X-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Ala-Leu-Gln

wherein X is cysteine or is not present.

5. The peptide of claim 1 or claim 2 wherein said peptide sequence comprises at least sixty percent of the amino acids found in the corresponding regions of said antigen and anti-idiotypic antibody, said corresponding regions are complementary determining regions of said anti-idiotypic antibody or said antibody specific for a cellular receptor of

said antigen and any sequence of said antigen and said peptides comprise at least six amino acids from corresponding regions of said antigen and said anti-idiotypic antibody or said antibody for a cellular receptor of said antigen.

6. A method of synthesizing biologically active compounds, comprising the steps of:

(a) selecting a pathogen gene product known to bind to a physiologic receptor of mammalian clls;

(b) providing an anti-idiotypic antibody for said pathogen gene product or an antibody for a cellular receptor of said pathogen gene product;

(c) comparing the peptide sequences of said gene product and of said anti-idiotypic antibody or said antibody for a cellular receptor of said pathogen gene product to determine at least shared portions thereof;

(d) snythesizing a compound comprising a biologically active site corresponding to the tertiary structure of said shared sequence portions.

7. The method of claim 5 further comprising the steps

(e) adding a connector to one end of a first peptide of step (d);

(f) adding a connector to one end of a second peptide of step (d);

(g) contacting said first and second peptides under conditions selected to allow association of said first and second peptides at said connector on each peptide to form peptide dimers.

8. The method of claim 5 or claim 6 wherein step (d) comprises synthesizing a peptide of claim 4.

9. The method of using combinations of complementary determining region peptides from the V_H and V_L regions to create antibody binding sites or antigens.

10. A method of altering the growth of a mammalian cell comprising administering to that cell a paptide comtrising a sequence of amino acids which is shared by a pathogen gene product known to bind to the beta adrenergic

receptor of said mammalian cell and by an anti-idiotypic antibody f r said pathogen or an antibody for the beta adgrnergic receptor, said sequence being selected and said peptide being administered in an amount effective to alter the growth of said cell.

11. A method of synthesizing biologically active compounds comprising the steps of:

(a) selecting a pathogen gene product known to bind to a physiologic receptor of mammalian cells;

(b) providing an anti-idiotypic antibody for said pathogen gene product;

(c) determining the amino acid sequence of said anti-idiotypic antibody in a complementary determining region of said antibody; and

(d) synthesizing a compound comprising a site corresponding to the tertiary structure of said complementary determining region of said anti-idiotypic anti-body.

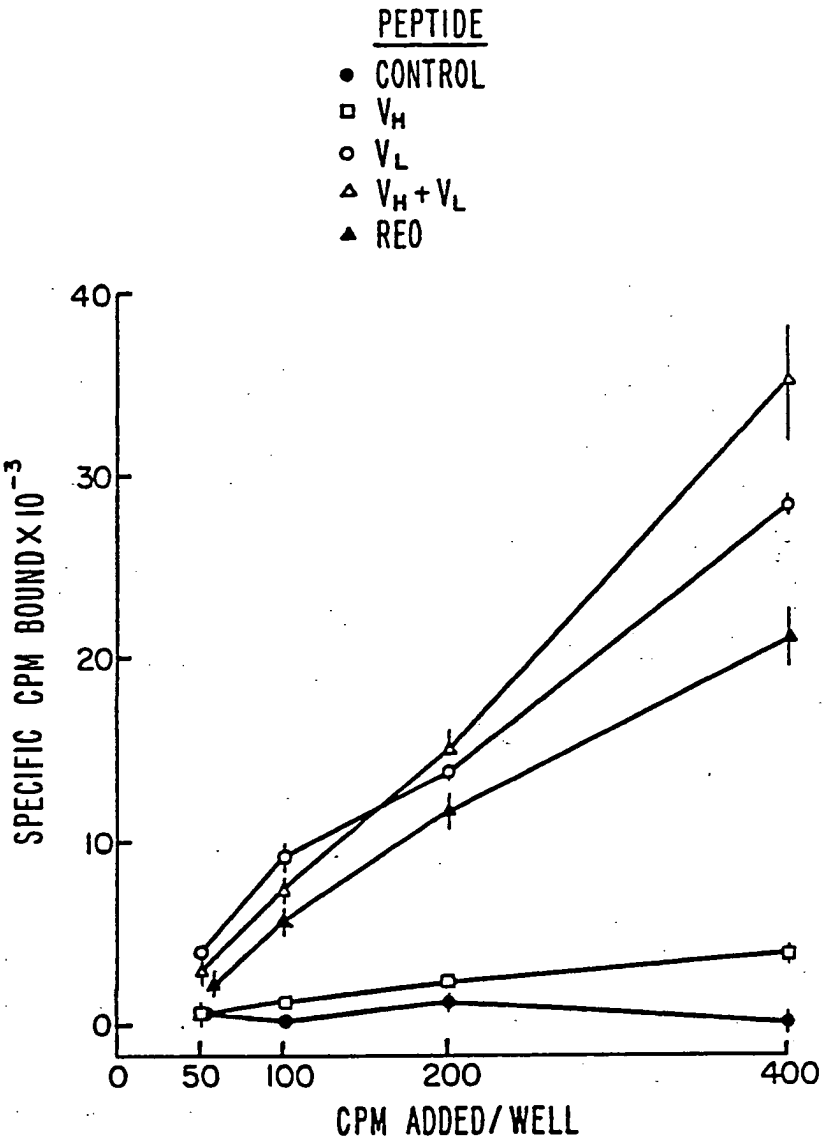

PEPTIDE
CONTROL
V_H
V_L
$V_H + V_L$
REO
SPECIFIC CPM BOUND X 10^{-3}
40
30
20
10
0
0
50
100
200
400
CPM ADDED/WELL

Fig. 1

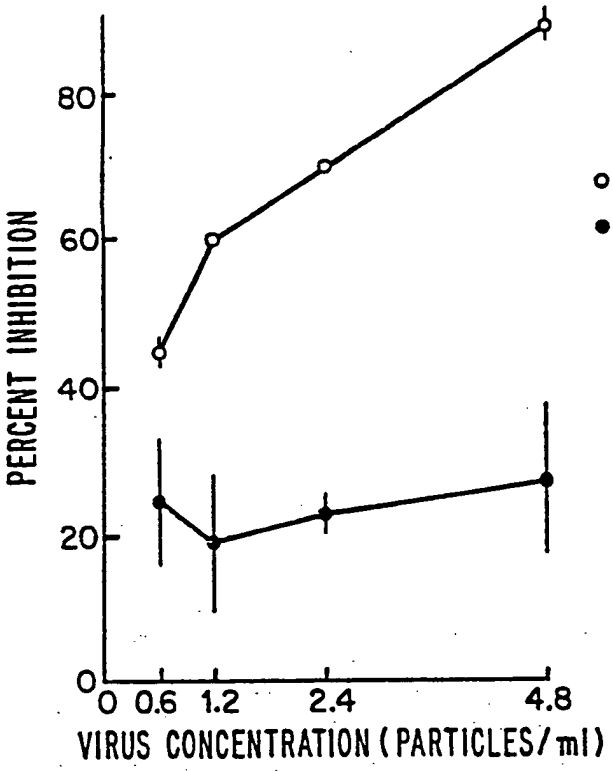

PERCENT INHIBITION
80
60
40
20
0
0 0.6 1.2 2.4 4.8
VIRUS CONCENTRATION (PARTICLES/ml)
INHIBITOR
o TYPE 3 VIRUS
• 3 HA1 VIRUS

Fig. 2

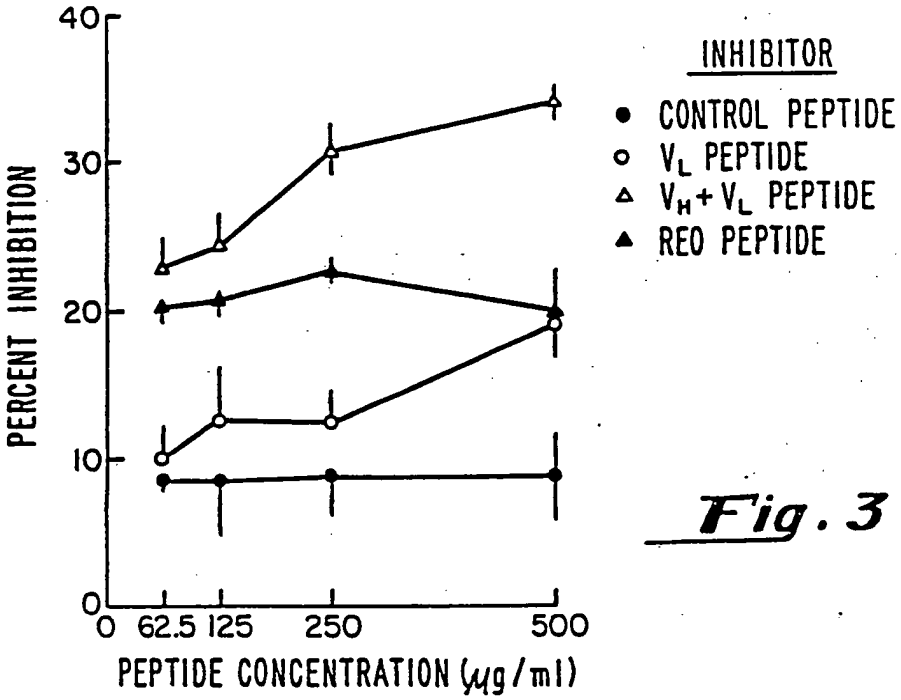

PERCENT INHIBITION
40
30
20
10
0
0 62.5 125 250 500
PEPTIDE CONCENTRATION (μg/ml)
INHIBITOR
• CONTROL PEPTIDE
o V_L PEPTIDE
△ $V_H + V_L$ PEPTIDE
▲ REO PEPTIDE

Fig. 3

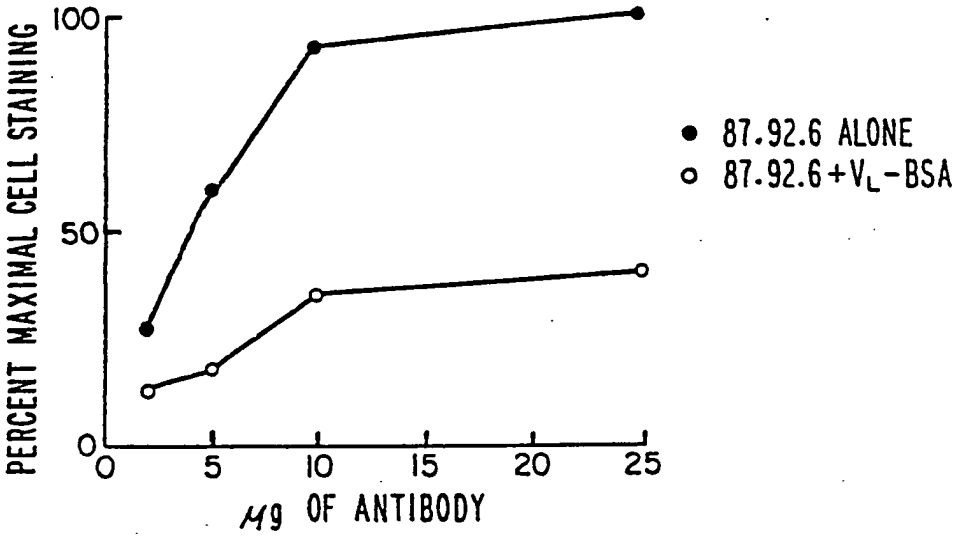

PERCENT MAXIMAL CELL STAINING
100
50
0
0
5
10
15
20
25
μg OF ANTIBODY
● 87.92.6 ALONE
○ 87.92.6+V_L-BSA

Fig. 4

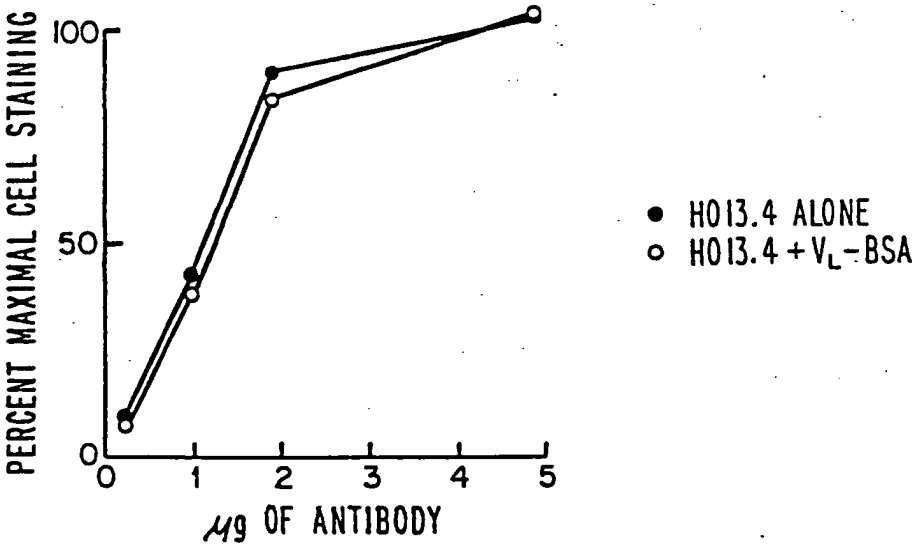

PERCENT MAXIMAL CELL STAINING
100
50
0
0
1
2
3
4
5
μg OF ANTIBODY
● HO13.4 ALONE
○ HO13.4+V_L-BSA

Fig. 5

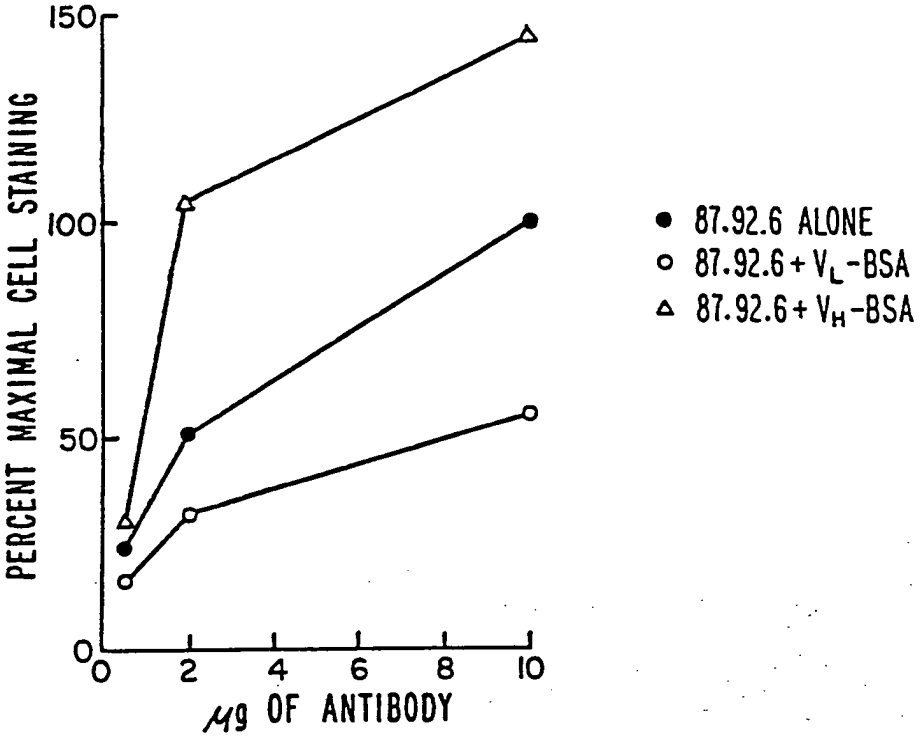


Fig. 6

TABLE 2

PERCENT POSITIVE

INHIBITOR	ANTIBODY ADDED	
EXPERIMENT 1	H013.4	87.92.6
NONE	99	60
V_H-BSA	99	67
V_L-BSA	99	26
EXPERIMENT 2		
NONE	99	85
V_L ALONE	92	80
$V_H + V_L$	88	33

Fig. 7

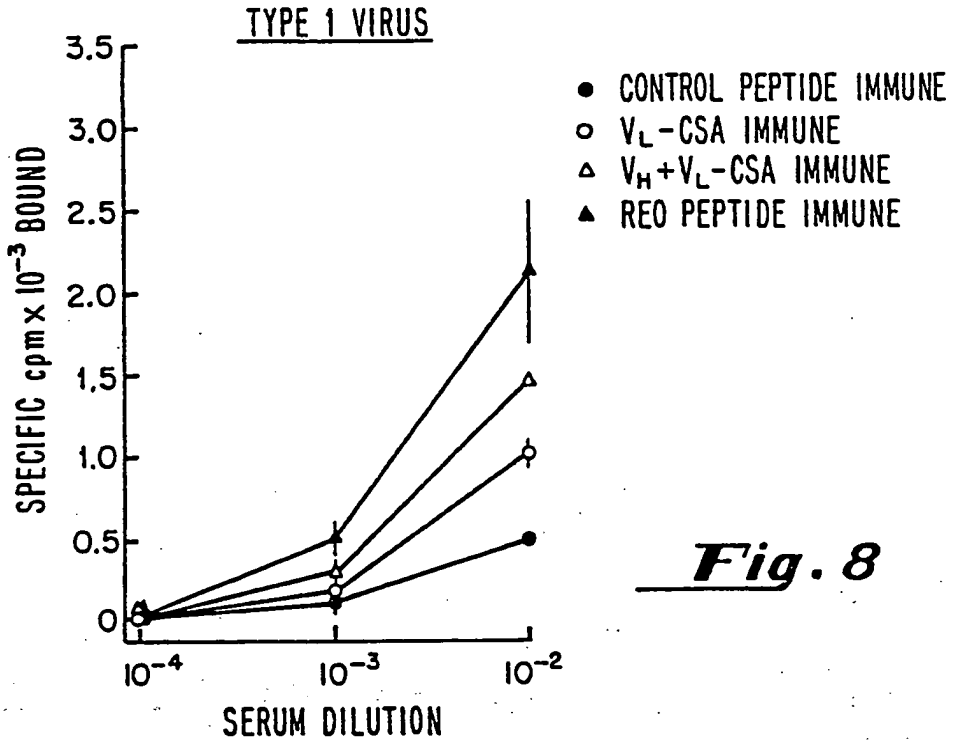

TYPE 1 VIRUS
SPECIFIC cpm x 10^{-3} BOUND
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10^{-4}
10^{-3}
10^{-2}
SERUM DILUTION
CONTROL PEPTIDE IMMUNE
V_L-CSA IMMUNE
V_H+V_L-CSA IMMUNE
REO PEPTIDE IMMUNE

Fig. 8

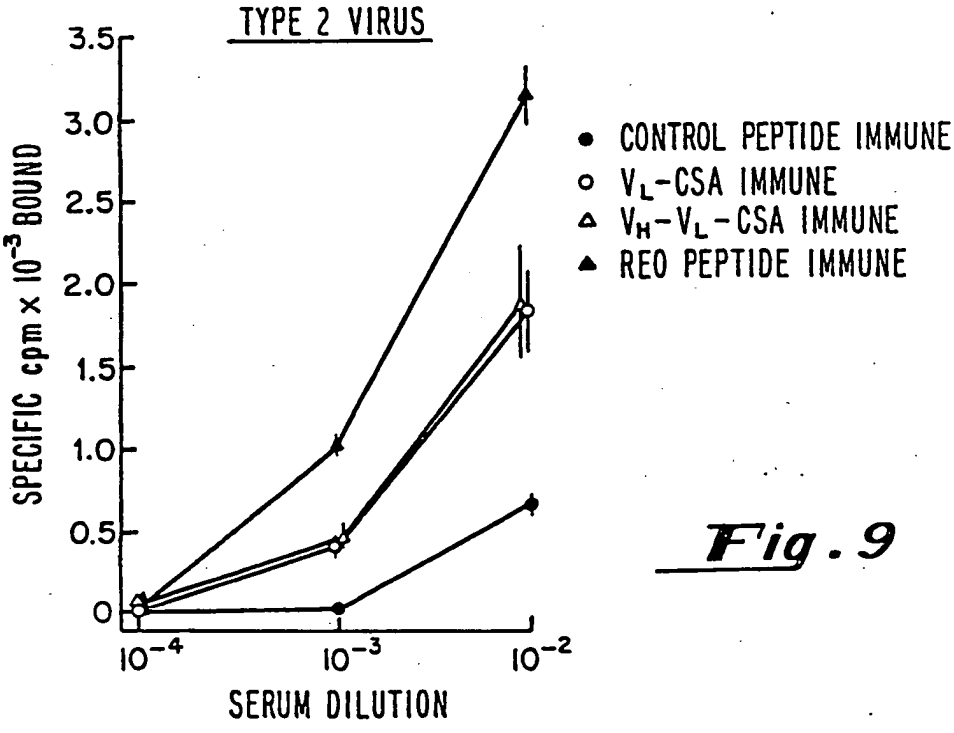

TYPE 2 VIRUS
SPECIFIC cpm x 10^{-3} BOUND
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10^{-4}
10^{-3}
10^{-2}
SERUM DILUTION
CONTROL PEPTIDE IMMUNE
V_L-CSA IMMUNE
V_H-V_L-CSA IMMUNE
REO PEPTIDE IMMUNE

Fig. 9

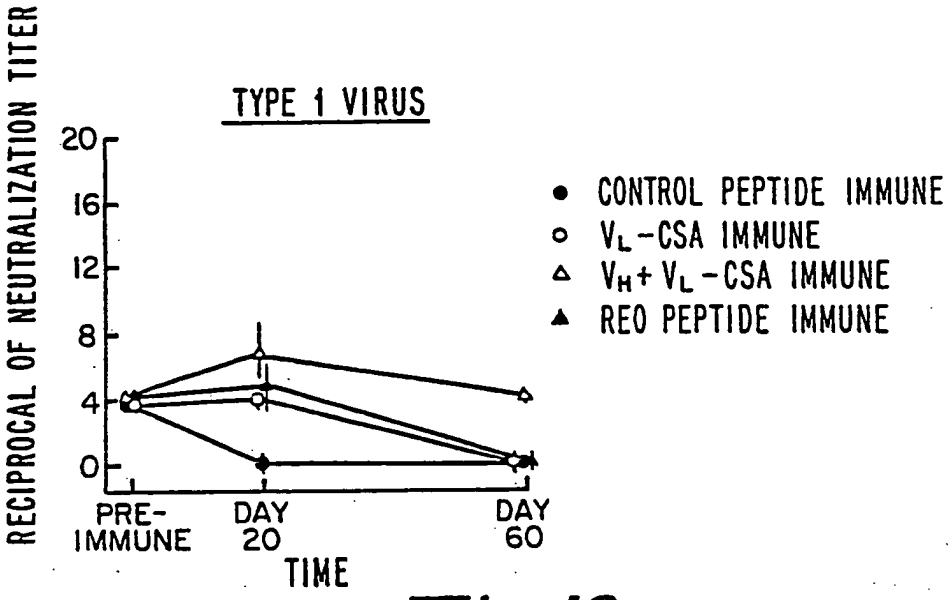

TYPE 1 VIRUS
RECIPROCAL OF NEUTRALIZATION TITER
20
16
12
8
4
0
PRE-IMMUNE
DAY 20
DAY 60
TIME
CONTROL PEPTIDE IMMUNE
V_L-CSA IMMUNE
V_H+V_L-CSA IMMUNE
REO PEPTIDE IMMUNE

Fig. 10

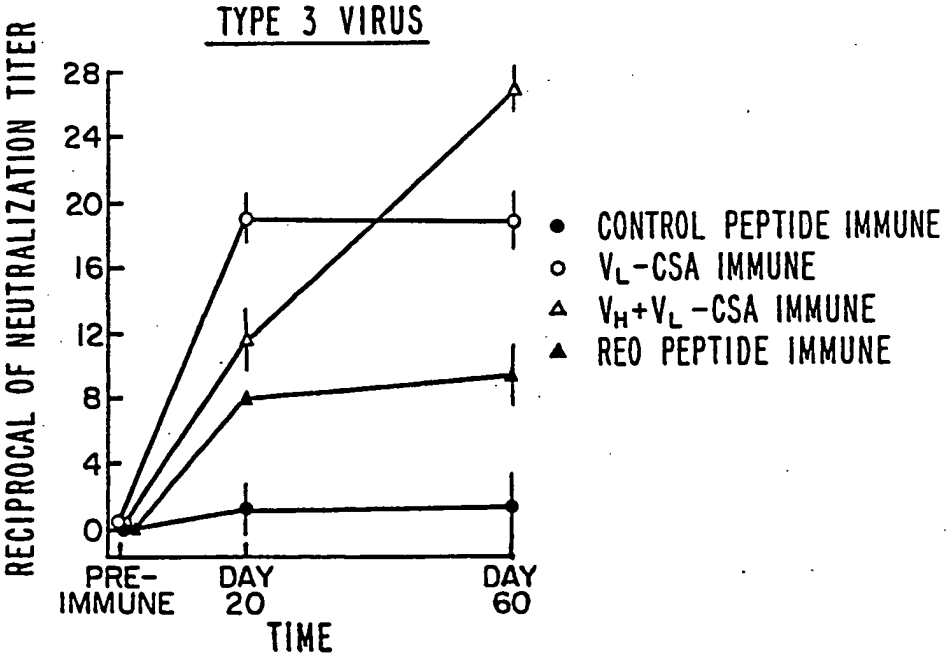

TYPE 3 VIRUS
RECIPROCAL OF NEUTRALIZATION TITER
28
24
20
16
12
8
4
0
PRE-IMMUNE
DAY 20
DAY 60
TIME
CONTROL PEPTIDE IMMUNE
V_L-CSA IMMUNE
V_H+V_L-CSA IMMUNE
REO PEPTIDE IMMUNE

Fig. 11

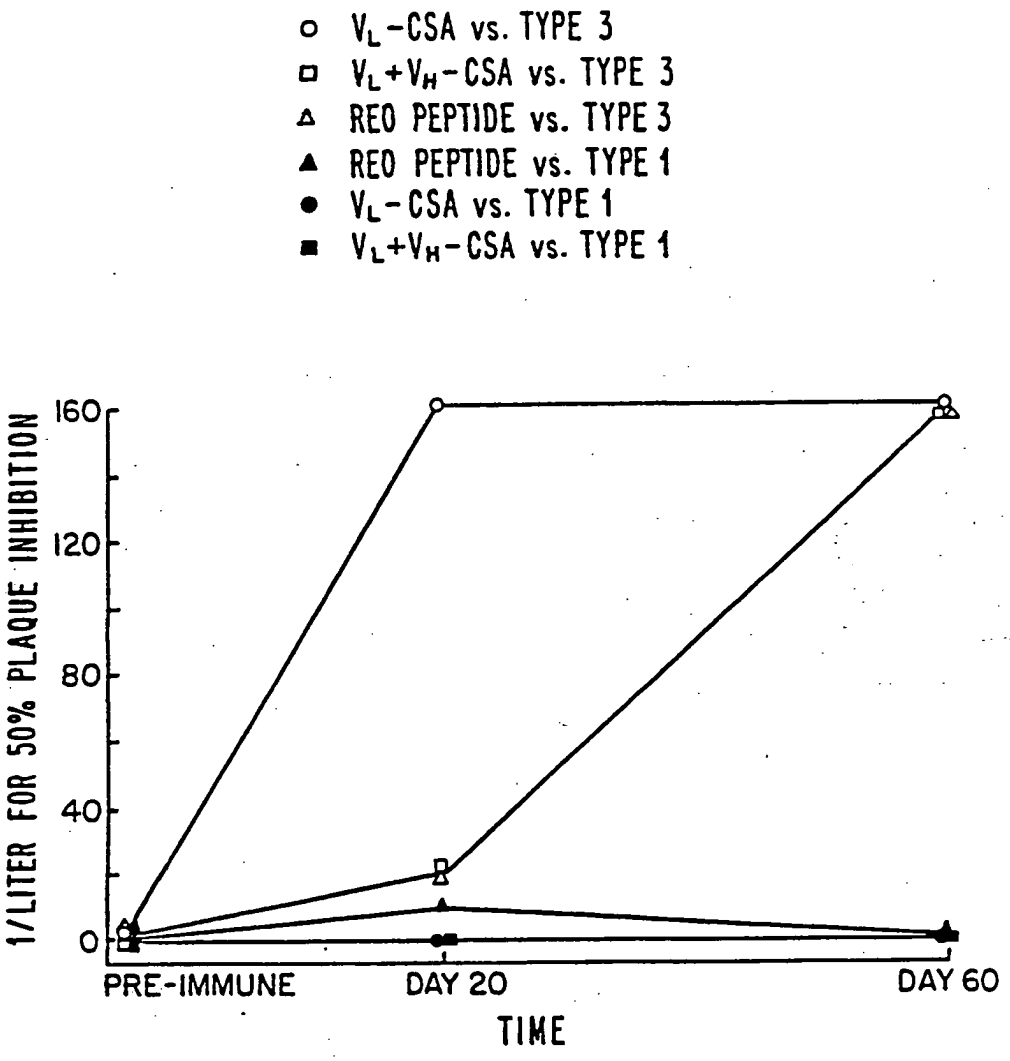
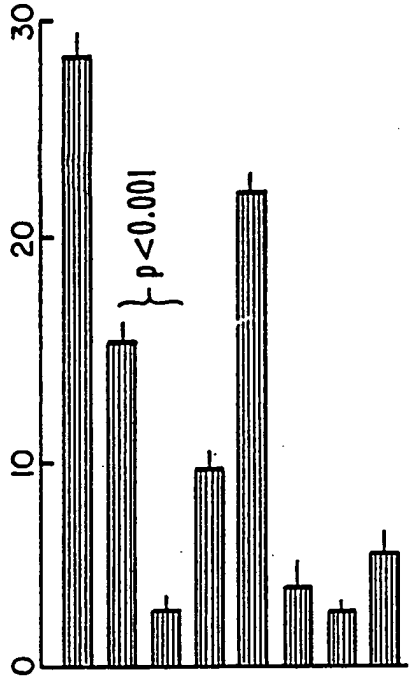
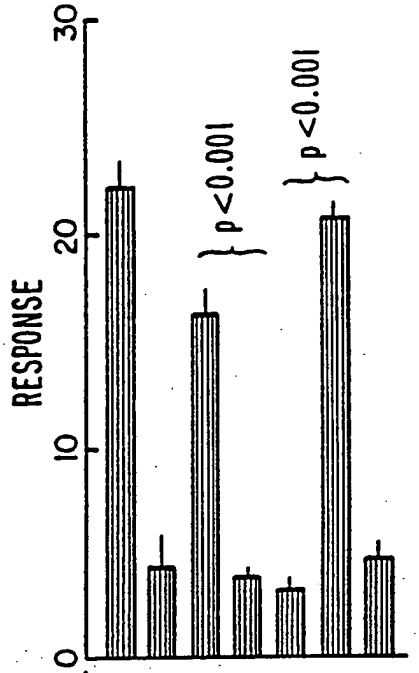

○ V_L-CSA vs. TYPE 3
□ V_L+V_H-CSA vs. TYPE 3
△ REO PEPTIDE vs. TYPE 3
▲ REO PEPTIDE vs. TYPE 1
● V_L-CSA vs. TYPE 1
■ V_L+V_H-CSA vs. TYPE 1
1/LITER FOR 50% PLAQUE INHIBITION
160
120
80
40
0
PRE-IMMUNE
DAY 20
DAY 60
TIME

Fig. 12



GROUP	IMMUNIZATION	CHALLENGE
I	1HA3	1HA3
II	1HA3	V_L PEPTIDE
III	1HA3	CONTROL PEPTIDE
IV	–	1HA3
V	3HA1	3HA1
VI	3HA1	V_L PEPTIDE
VII	3HA1	CONTROL PEPTIDE
VIII	–	3HA1

Fig. 13



GROUP	IMMUNIZATION	CHALLENGE
I	1HA3	1HA3
II	–	1HA3
III	V_L PEPTIDE	1HA3
IV	CONTROL PEPTIDE	1HA3
V	V_L PEPTIDE	3HA1
VI	3HA1	3HA1
VII	–	3HA1

Fig. 14

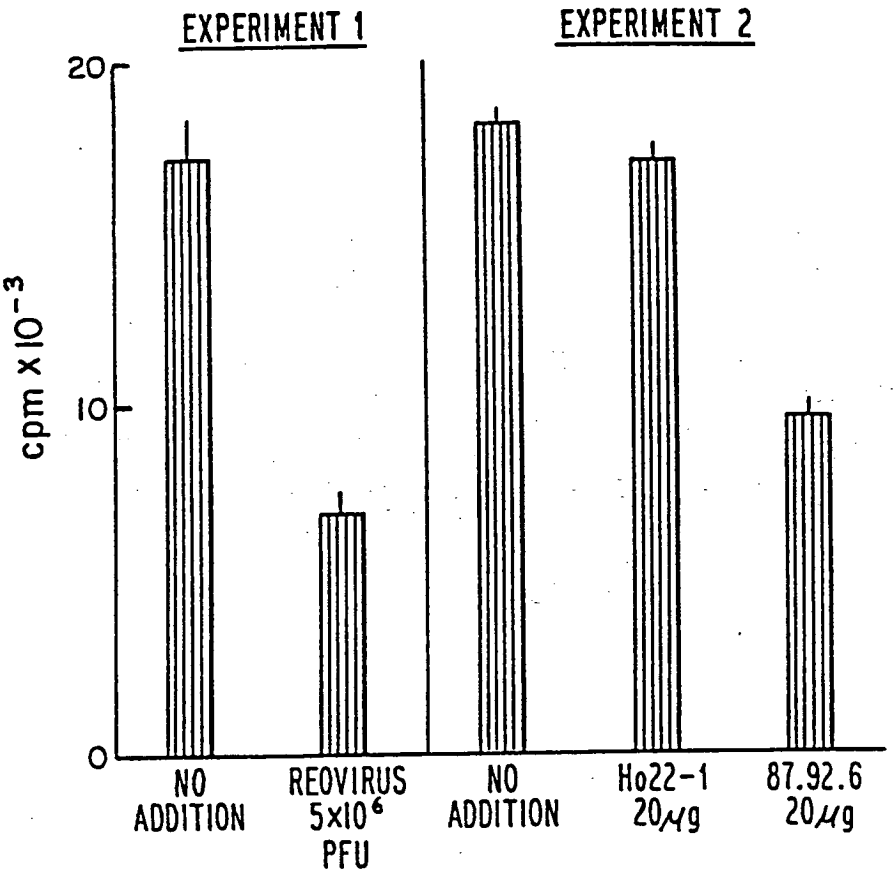

EXPERIMENT 1
EXPERIMENT 2
20
10
0
cpm x 10⁻³
NO ADDITION
REOVIRUS 5x10⁶ PFU
NO ADDITION
Ho22-1 20μg
87.92.6 20μg

Fig. 15

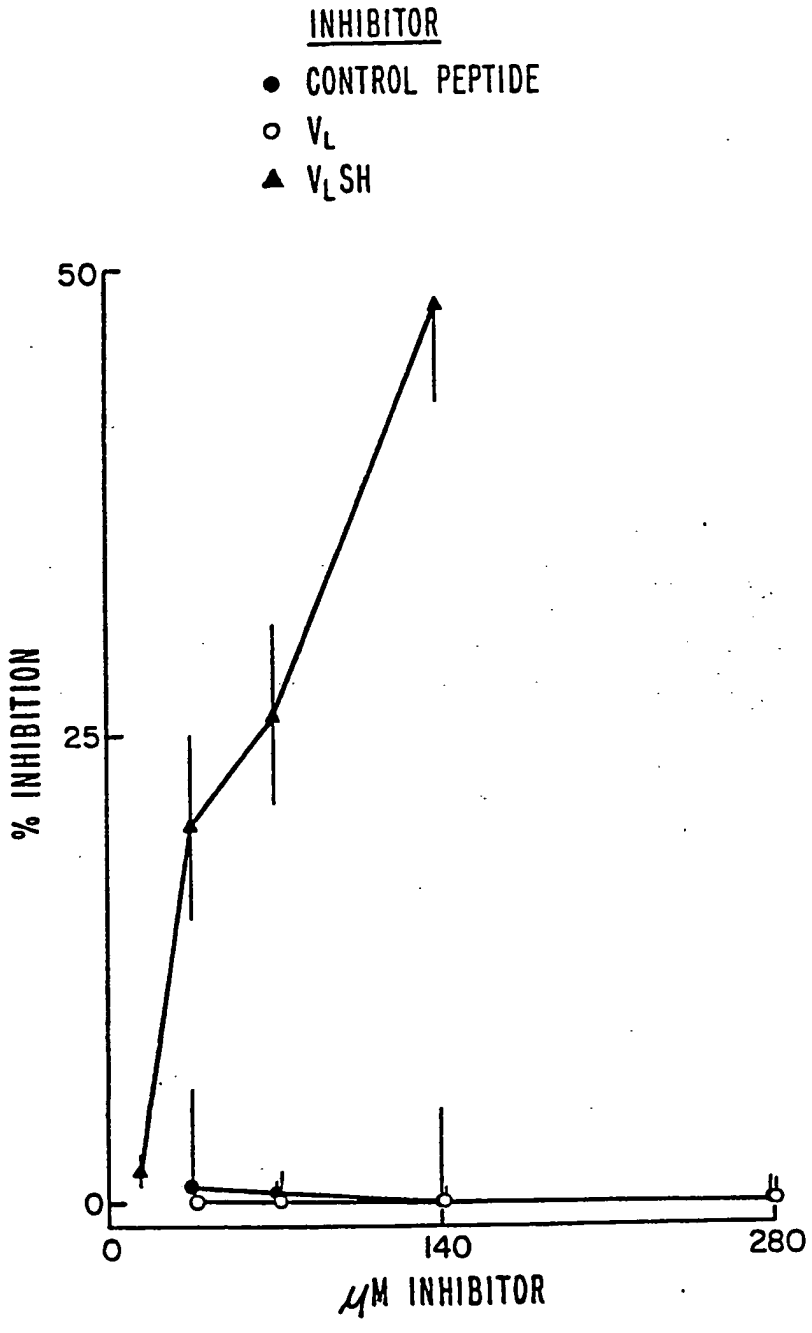

INHIBITOR
CONTROL PEPTIDE
V_L
V_LSH
50
25
0
% INHIBITION
0
140
280
μM INHIBITOR

Fig. 16

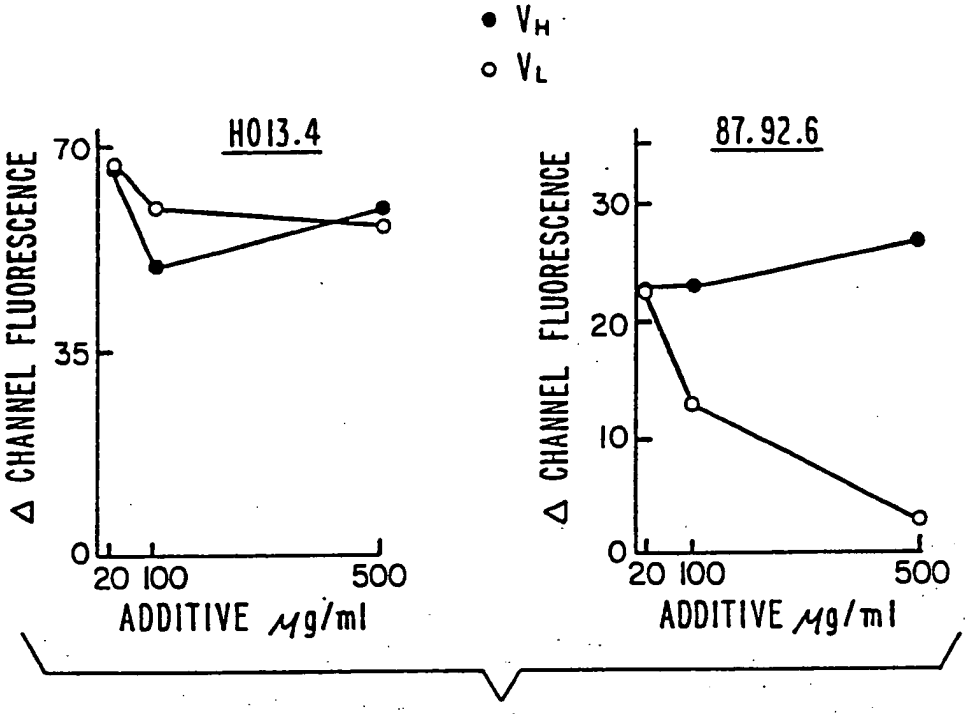

• V_H
○ V_L
H013.4
87.92.6
Δ CHANNEL FLUORESCENCE
70
35
0
30
20
10
20 100 500
ADDITIVE μg/ml

Fig. 17

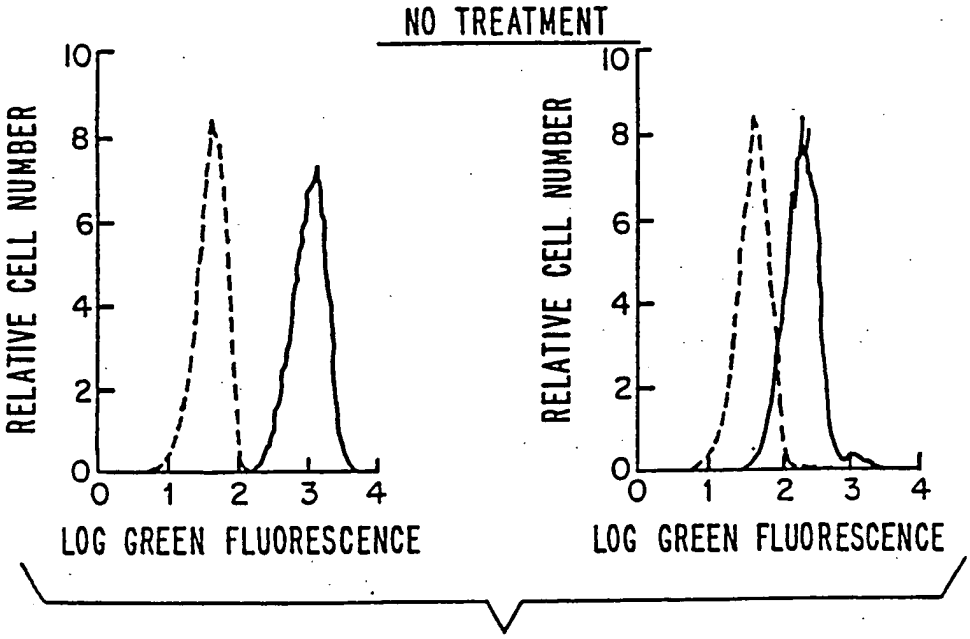

NO TREATMENT
RELATIVE CELL NUMBER
10
8
6
4
2
0
0 1 2 3 4
LOG GREEN FLUORESCENCE

Fig. 18

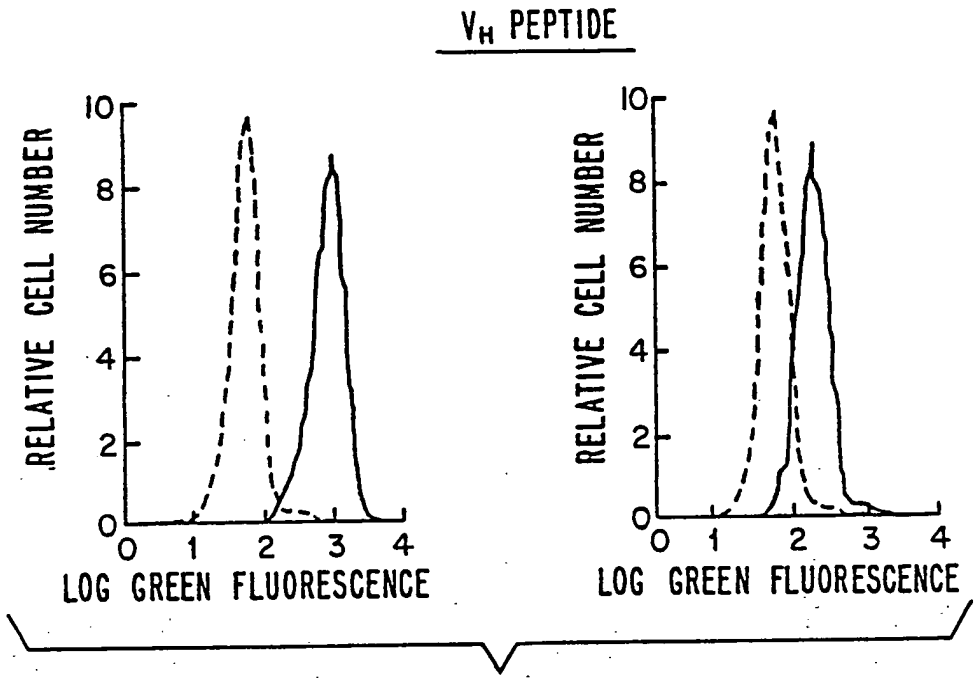

V_H PEPTIDE
RELATIVE CELL NUMBER
LOG GREEN FLUORESCENCE
RELATIVE CELL NUMBER
LOG GREEN FLUORESCENCE

Fig. 19

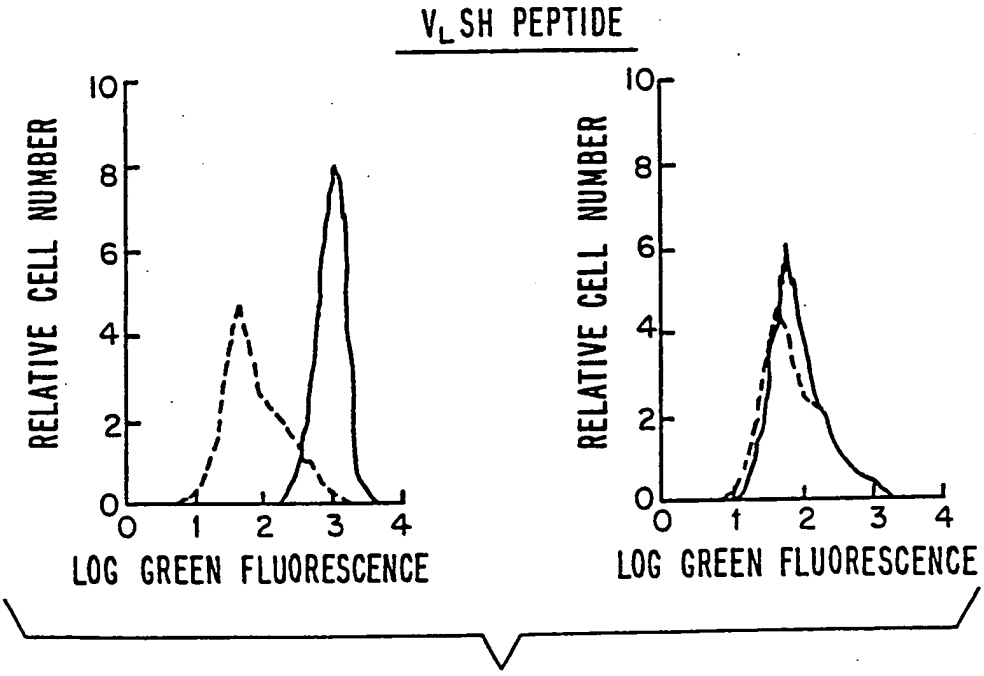

V_LSH PEPTIDE
RELATIVE CELL NUMBER
LOG GREEN FLUORESCENCE
RELATIVE CELL NUMBER
LOG GREEN FLUORESCENCE

Fig. 20

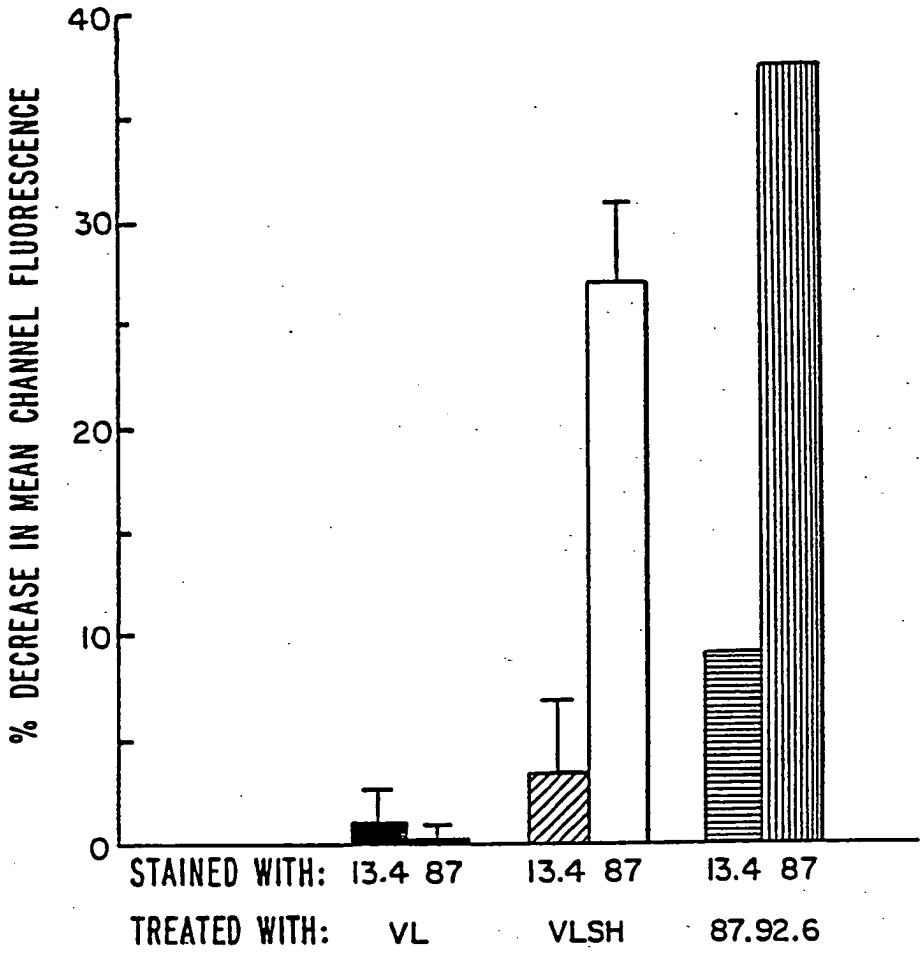

% DECREASE IN MEAN CHANNEL FLUORESCENCE
40
30
20
10
0
STAINED WITH: 13.4 87 13.4 87 13.4 87
TREATED WITH: VL VLSH 87.92.6

Fig. 21

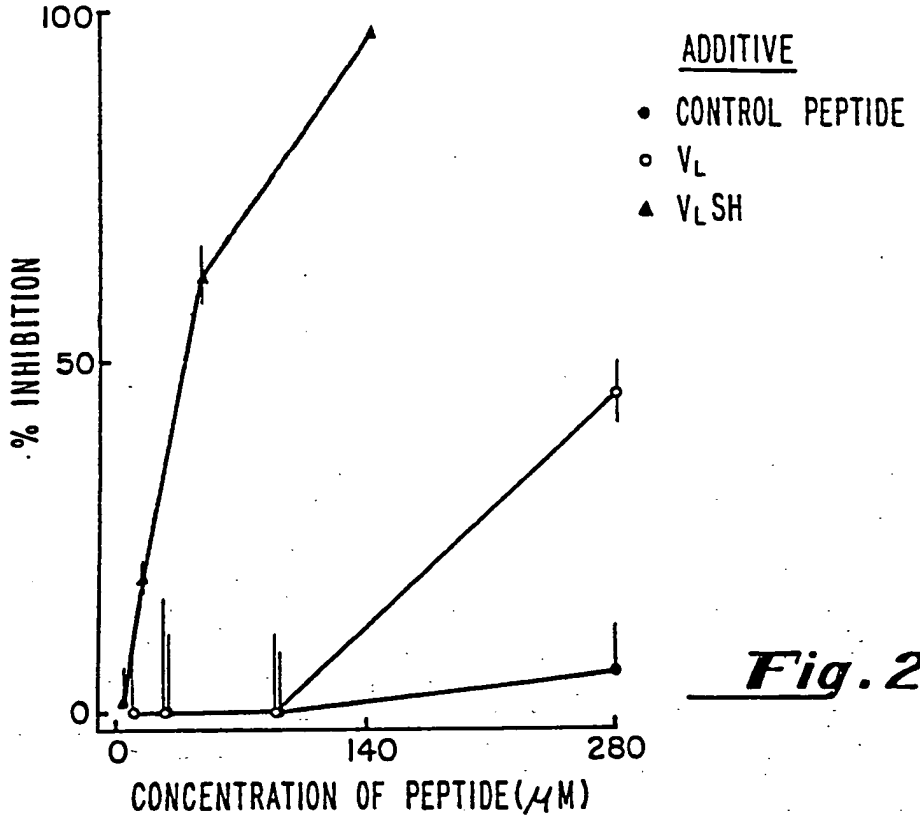

100
50
0
% INHIBITION
0
140
280
CONCENTRATION OF PEPTIDE(μM)
ADDITIVE
• CONTROL PEPTIDE
○ V_L
▲ V_LSH

Fig. 22

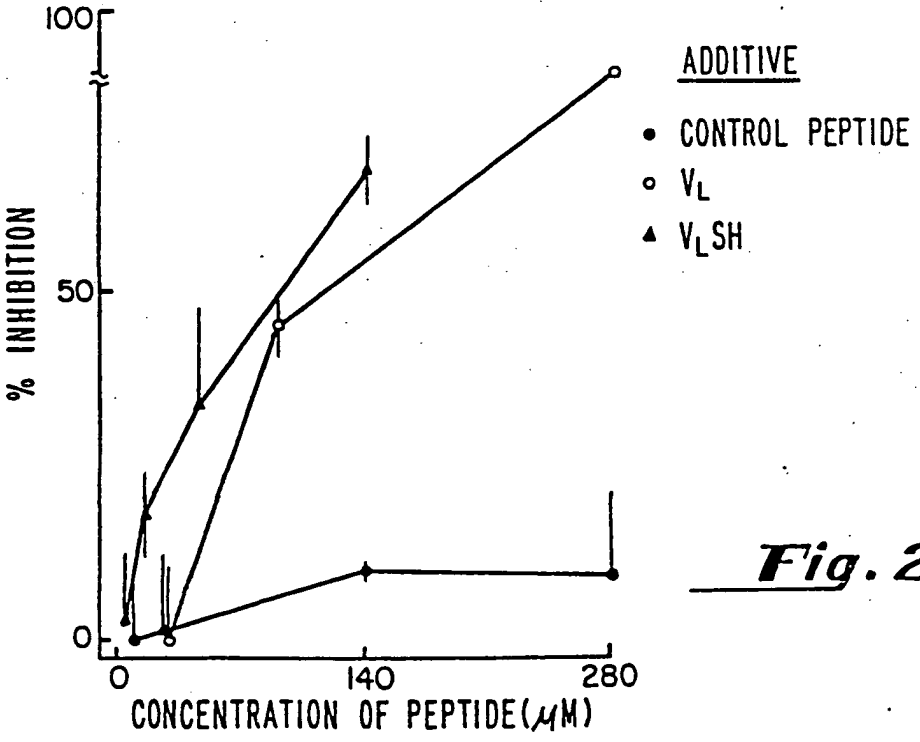

100
50
0
% INHIBITION
0
140
280
CONCENTRATION OF PEPTIDE(μM)
ADDITIVE
• CONTROL PEPTIDE
○ V_L
▲ V_LSH

Fig. 23

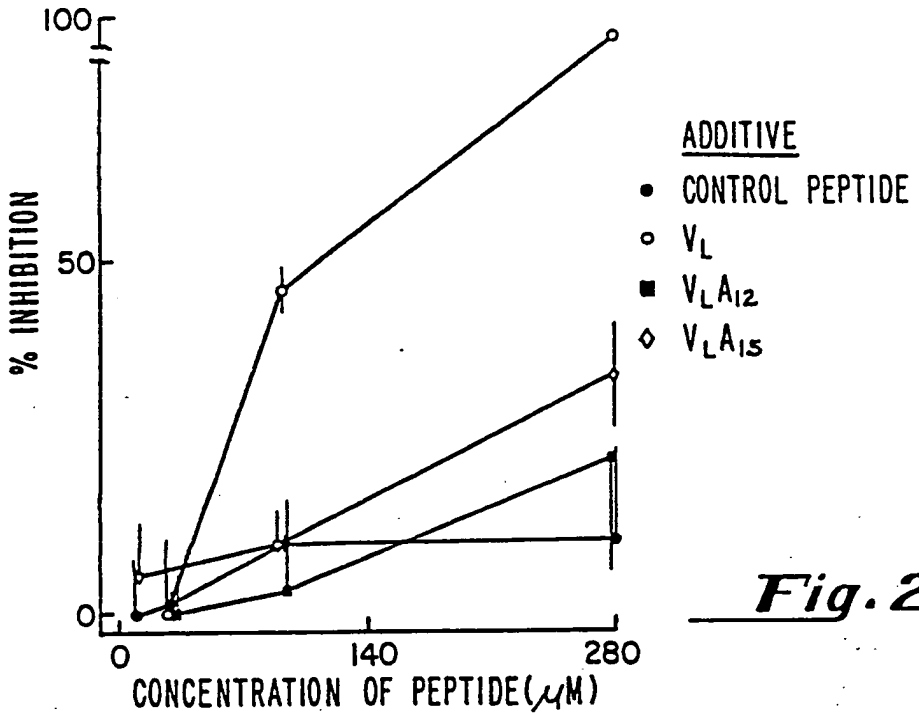

100
50
0
% INHIBITION
0
140
280
CONCENTRATION OF PEPTIDE(μM)
ADDITIVE
CONTROL PEPTIDE
V_L
V_LA_{12}
V_LA_{15}

Fig. 24

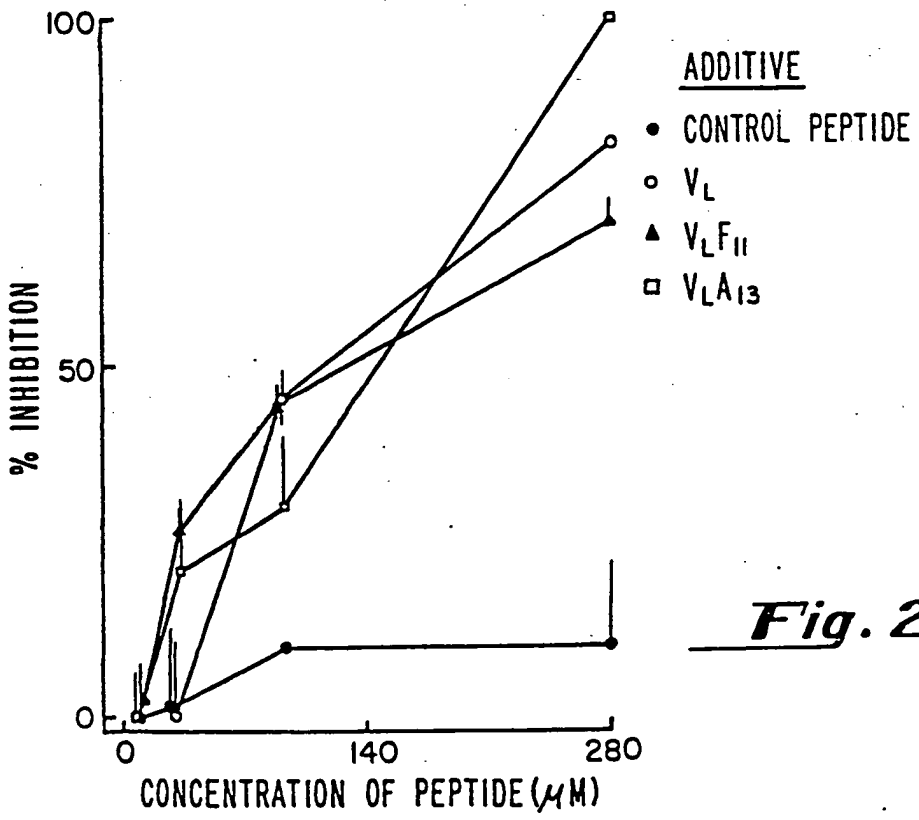

100
50
0
% INHIBITION
0
140
280
CONCENTRATION OF PEPTIDE(μM)
ADDITIVE
CONTROL PEPTIDE
V_L
V_LF_{11}
V_LA_{13}

Fig. 25

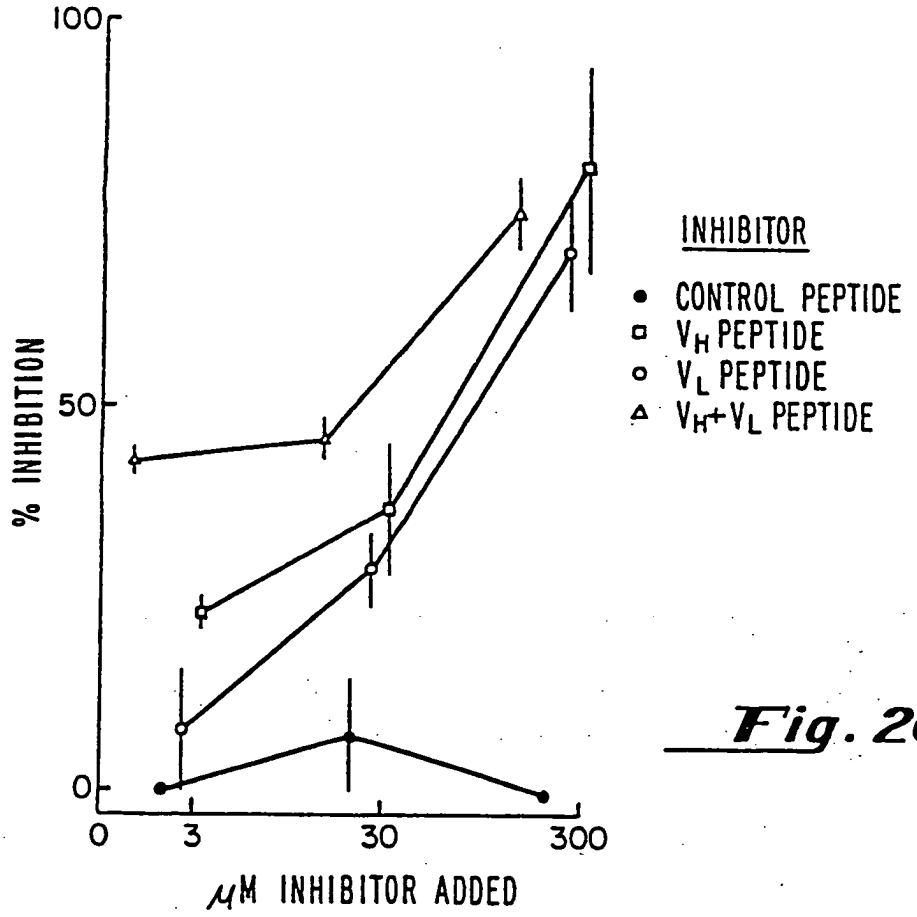

100
50
0
% INHIBITION
0
3
30
300
μM INHIBITOR ADDED
INHIBITOR
CONTROL PEPTIDE
V_H PEPTIDE
V_L PEPTIDE
V_H+V_L PEPTIDE

Fig. 26

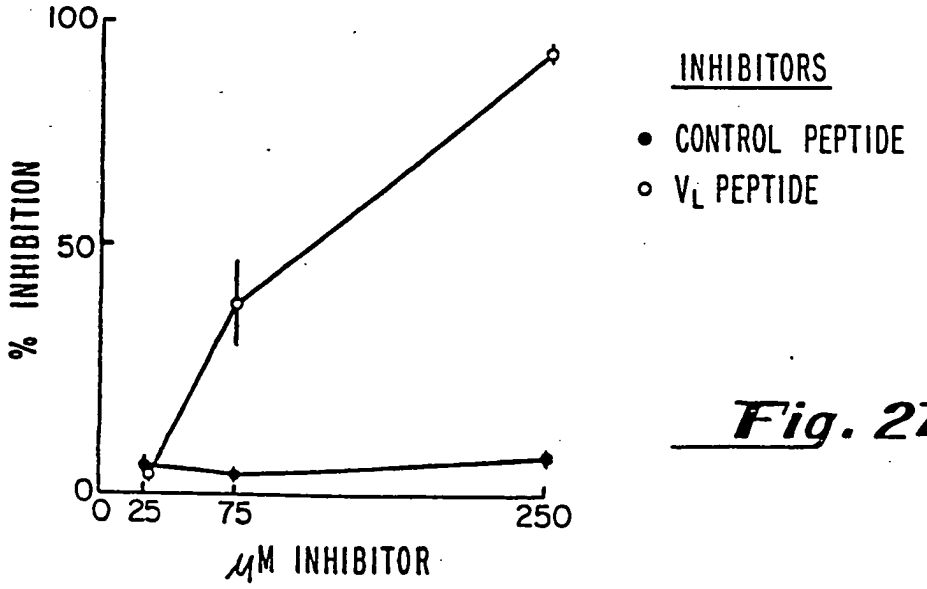

100
50
0
% INHIBITION
0
25
75
250
μM INHIBITOR
INHIBITORS
CONTROL PEPTIDE
V_L PEPTIDE

Fig. 27

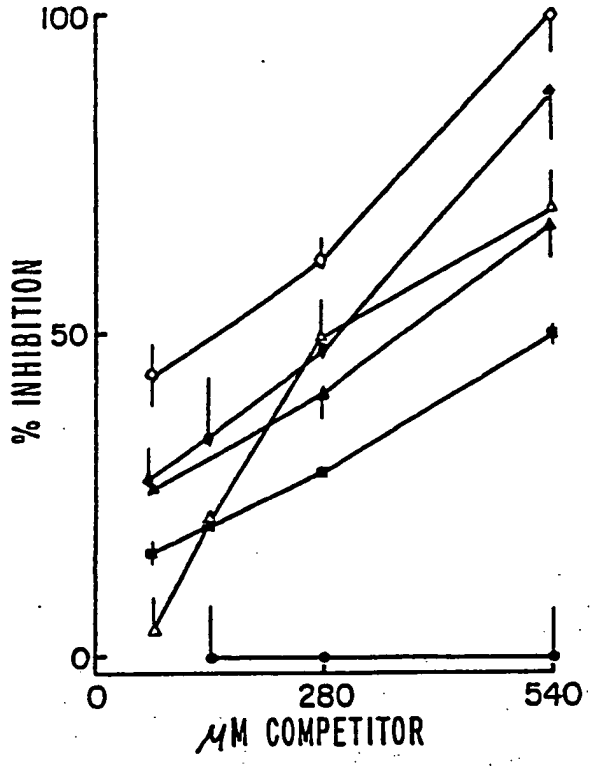

100
50
0
% INHIBITION
0
280
540
μM COMPETITOR
COMPETITOR
CONTROL PEPTIDE
V_LA_6
V_LF_{11}
V_LA_{12}
V_LA_{14}
V_LA_{15}

Fig. 28

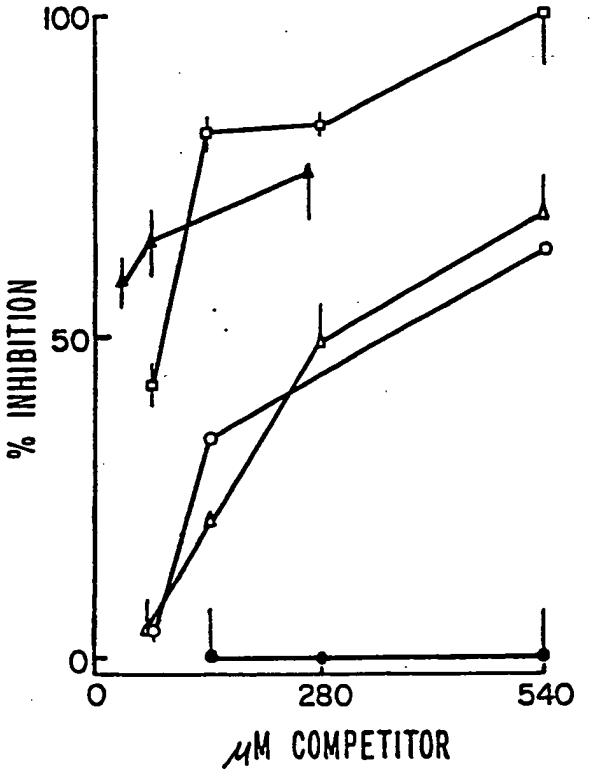

100
50
0
% INHIBITION
0
280
540
μM COMPETITOR
COMPETITOR
CONTROL PEPTIDE
V_L
V_LA_6
V_LA_{13}
V_LSH

Fig. 29

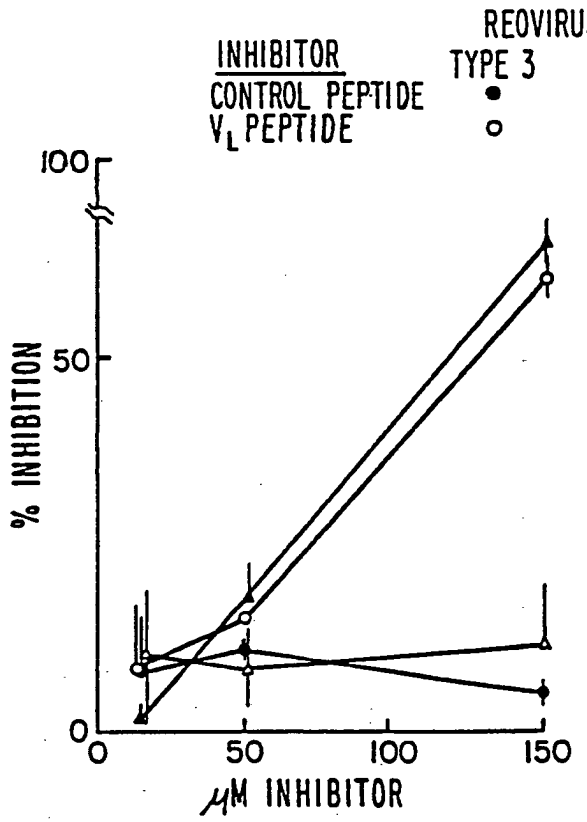

REOVIRUS TYPE
INHIBITOR
TYPE 3
VARIANT K
CONTROL PEPTIDE
V_L PEPTIDE
100
50
0
% INHIBITION
0
50
100
150
μM INHIBITOR

Fig. 30

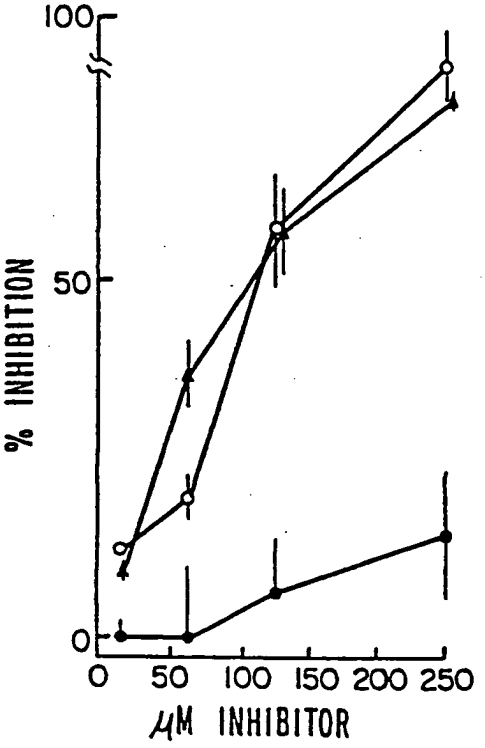

100
50
0
% INHIBITION
0
50
100
150
200
250
μM INHIBITOR
REOVIRUS 1
REOVIRUS 3
REOVIRUS VARIANT K

Fig. 31

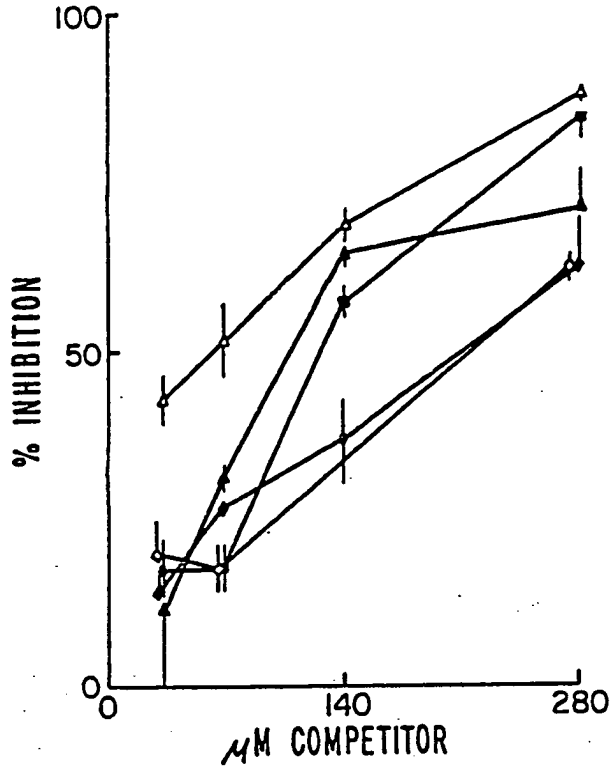

100
50
0
% INHIBITION
0
140
280
μM COMPETITOR
COMPETITOR
V_LA_6
V_LF_{11}
V_LA_{12}
V_LA_{14}
V_LA_{15}

Fig. 32

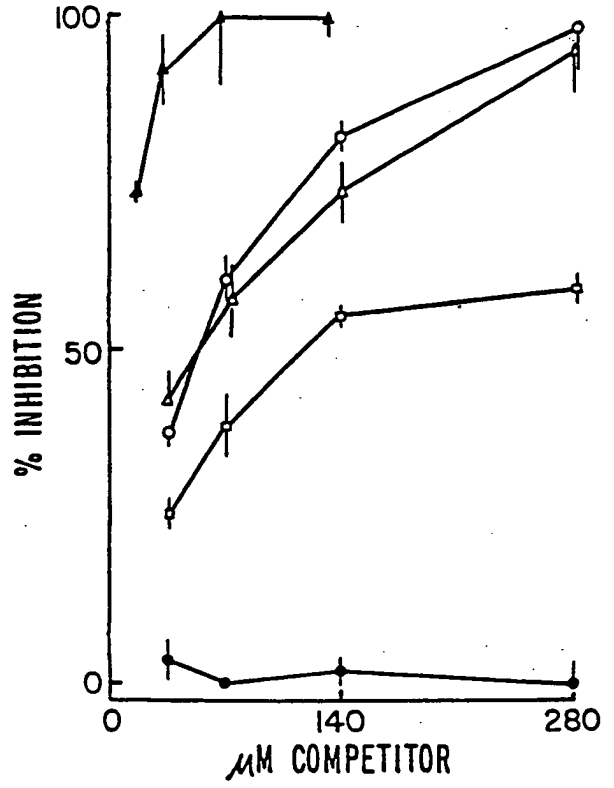

100
50
0
% INHIBITION
0
140
280
μM COMPETITOR
COMPETITOR
CONTROL PEPTIDE
V_L
V_LA_6
V_LSH
V_LA_{13}

Fig. 33

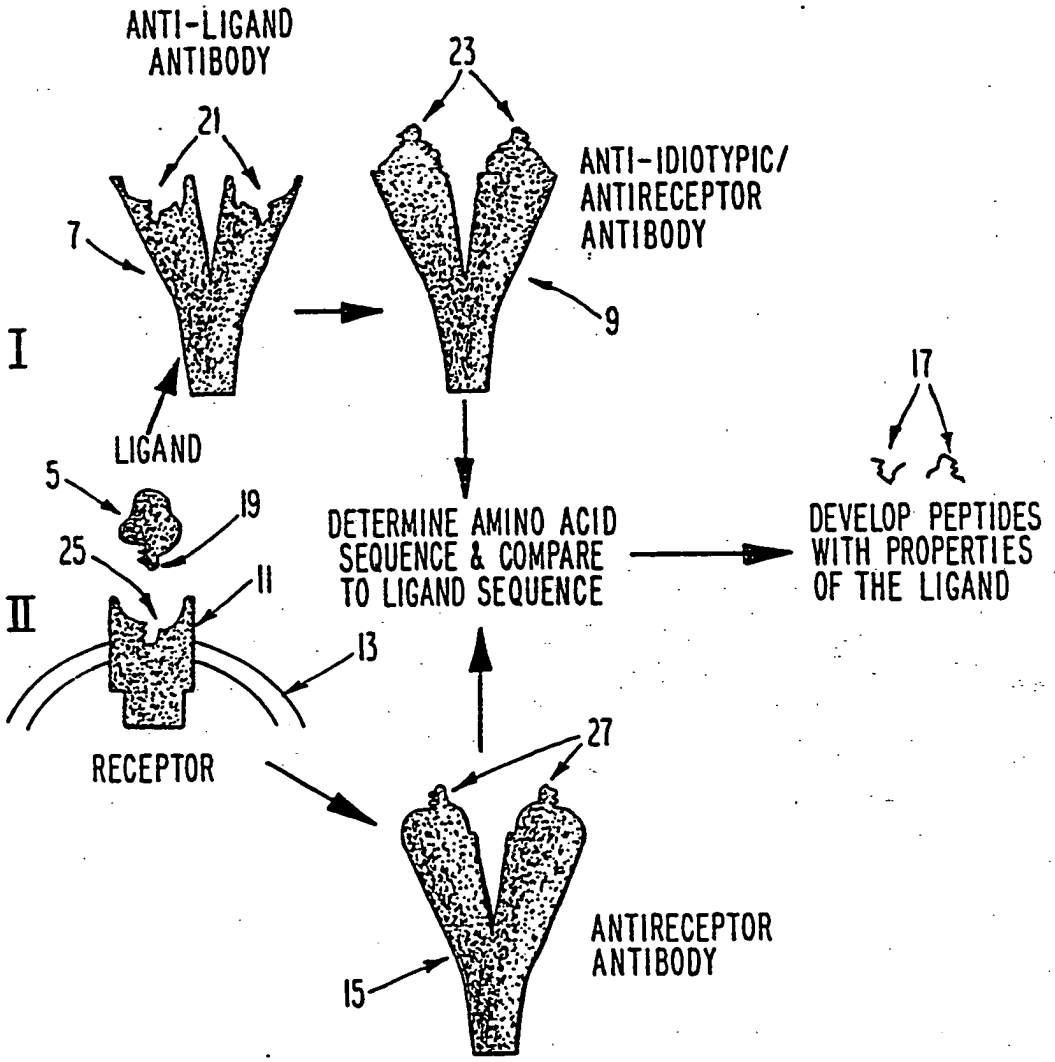

ANTI-LIGAND ANTIBODY
21
23
ANTI-IDIOTYPIC/ ANTIRECEPTOR ANTIBODY
7
9
I
LIGAND
17
5
19
DETERMINE AMINO ACID SEQUENCE & COMPARE TO LIGAND SEQUENCE
DEVELOP PEPTIDES WITH PROPERTIES OF THE LIGAND
25
11
II
13
RECEPTOR
27
ANTIRECEPTOR ANTIBODY
15

Fig.34

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/01815

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

C07K 15/00, 5/02, 7/00, 1/00, 15/00, C12P 21/00;
A61K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/68; 514/12,13; 530/300, 323, 326, 333, 350, 402

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

DIALOG DATABASE FILES: 5, 10, 155, 238, 350-1, 357-8, 399 (Palo Alto, CA) Automated Patent System (APS). Search date 13 JUL 1989.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	US, A, 4,683,295 CARSON 28 JUL 1987 See: abstract.	1, 6, 11 1-3, 5-11
P,Y	US, A, 4,761,371 BELL 02 AUG 1988 See: abstract.	1-3, 5-11
Y	WO, A, 8702990 SCHERING-BIOTECH CO. 21 MAY 1987. See abstract.(DIALOG DATABASE FILE 351, WPI Acc. No: 87-150613/21. XRAM Acc No: C87-062883.)	1-3, 5-11
PY	WO, A, 8807375 HIVER, 06 OCT 1988. See abstract. (DIALOG database file: 357, WPI Acc. No: 88-292708.)	1-3, 5-11
PX PY	WO, A, 8809181 TANOX BIOSYSTEMS IN. 01 DEC 1988. See abstract. (DIALOG DATABASE FILE: 351, WPI Acc. No. 88-353807/49. XRAM Acc. No. C88-156503.)	1, 6, 11 1-3, 5-11
Y	EP, A, 241139 MERCK AND CO INC 14 OCT 1987. See abstract. (DIALOG DATABASE FILE: 351, WPI Acc. No. 87-285998/41, XRAM Acc. No. C87-121247.)	1-3, 5-11
Y	Journal of Immunology Vol. 128, pp. 247-250. JAN 1982. R. B. Fritz et. al. " Idiotypes of Lewis Rat antibodies. . ." See abstract and p. 250.	1-3, 5-11

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 JUL 1989	11SEP 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Thomas M. Cunningham, Ph.D

FURTHER INFORMATI N CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1].

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Group I: claims 1, 4, 5, 6, 8-9, and 11 drawn to linear peptides. . .
Group II:claims 2, 3, 7, and 10 drawn to peptide dimers.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable clai of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers o those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (C NTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X Y	Journal of Immunology Vol. 136, pp. 582-7 JAN 1986 M. V. Seiden, "Hypervariable Region Peptides Variably Induce Specific Anti-idiotype Antibodies. . ." See: abstract and p. 586.	1, 9 1-3, 5-11
Y	Journal of Immunology Vol. 140, pp. 3059-65 MAY 1988 M. Budisavljevic, et. al. "Angiotensin II (AII)-Related Idiotypic Network. See: abstract and p. 3064.	1-3, 5-11
Y	R. A. Lerner, et. al. (editors), Vaccines 85, Cold Spring Harbor Laboratory, New York 11724, published 1985. See: pp. 151-156.	1-3, 5-11
Y	Proceedings of the National Academy of Sciences, Vol. 84, pp. 3891-3895. JUN 1987, T. C. Chanh, et. al. "Monoclonal Anti-idiotypic Antibody Mimics the CD4 Receptor and Binds Human Immunodeficiency Virus." See: abstract and p. 3895.	1-3, 5-11
X	Proceedings of the National Academy of Sciences, Vol. 83, pp. 6578-6582. SEP 1986, C. Bruck, et. al. "Nucleic Acid Sequence of an Internal Image-bearing Monoclonal Anti-idiotype and its Comparison to he Sequence of theExternal Antigen. See: abstract and p. 6582.	1-3, 5-9, 11
X Y	Journal of Experimental Medicine, Vol 164, pp.227-236. JUL 1986. Y. M. Thanaval, et. al. "A Surrogate Hepatitis B Virus Antigenic Epitope Represented by a Synthetic Peptide and an Internal Image Anti-idiotype Antibody. See: summary and p. 235.	1, 6, 11 1-11
Y	Cancer Research, Vol. 45, pp.6119-6123, DEC 1985, L. J. Smith, et. al. "Production of Heterologous Antibodies for Murine B-cell Leukemia Immunoglobulin by Immunization withSynthetic Peptides Homologous to Heavy Chain Hypervariable Regions. See: abstract and pp. 6122-3.	1, 9
Y	Molecular and Cellular Biochemistry, Vol 65, pp. 5-21, NOV 1984, C. N. Gaulton, et. al. "Anti-idiotypic Antibodies as Probes of Cell Surface Receptors." See: abstract as cited in DIALOG DATABASE FILE 155, Acc. number 85110880.	1-3, 5-11
A	R. Arnon (editor), Synthetic Vaccines, Vol. 1, 1987, CRC Press, Boca Raton Florida. See abstract as cited in DAILOG DATABASE FILE 5, BIOSIS No. 35080391.	1-11